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TWO NEW SPECIES OF MONOSTOMES FROM THE CANADA GOOSE WITH A REVIEW OF *PARAMONOSTOMUM ALVEATUM* (MEHLIS IN CREPLIN, 1846)

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On March 8, 1950 a Canada goose, *Branta canadensis,* was found in the water near the Bellamy River bridge (Great Bay) at Durham, New Hampshire. Examination yielded no indication of the cause of death although large numbers of trematode parasites were recovered from the intestine and ceca. No further pathological investigation was made. Study of the parasites indicated that they were representatives of the subfamily NOTOCOTYLINAE. Detailed observations on a long series of these helminths led to the conclusion that two new species were represented.

Worms used in this study were fixed in alcohol-formalin-acetic acid and stained in a 1 : 9 dilution of Ehrlich's hematoxylin or a modified Reynolds' stain consisting of 1 part Ehrlich's hematoxylin, 3 parts alum cochineal, 4 parts 95% alcohol, and 8 parts of distilled water. Longitudinal and transverse sections were made and stained in Ehrlich's hematoxylin, Mallory's triple stain (after post-Zenkerization), safranin and fast green, and Goldman's iron alum-picric acid-hematoxylin.

Preliminary studies were made on living specimens. Eggs passed from the uterus of the parasites were placed in dishes containing *Physa heterostropha* from a nearby pond and *Helisoma sp.* from a biological supply house. Upon examination six weeks later these snails were negative for trematode infection.

GENUS *Catatropis* ODHNER, 1905

Harwood (1939) reviewed the genus *Catatropis* and emphasized the need for a stricter adherence to the original generic concept of Odhner (1905). The most important aspect of Odhner's concept concerned the pattern of the ventral glands as compared to the other members of the NOTOCOTYLINAE. This pattern in *Catatropis* characteristically comprises a median glandular ridge and two weakly developed lateral rows of glands that are always protruded. Other characters, such as size of cirrus sac, are not only unreliable but even misleading.

In the present study, about 1000 worms of this genus were recovered from the ceca of the Canada goose. Of these, 150 specimens were studied as stained whole mounts and 14 were sectioned for more detailed study. Measurements indicated that this was a hitherto undescribed species which is here described as *Catatropis harwoodi.*

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CATATROPIS HARWOODI, N. SP.
(figs. 1, 2 and 3)

The body is elongate and much flattened dorsoventrally. The anterior end is pointed while the posterior region is rounded. The lateral edges of the anterior portion of the body project ventrally, producing a pronounced depression in that region. Mature specimens varied from 2.0 to 3.5 mm. in length and from 0.5 to 0.8 mm. in width. The integument of the ventral surface of the anterior body third is thickly covered with small spines. Ventral integumentary glands are present in the arrangement characteristic of the genus *Catatropis*. There is a median, ventral glandular ridge extending from the level of the middle of the testes to the region just behind the cirrus sac. Lateral to this ridge on either side is a row of 7 to 9 small glands that extend from the level of the ovary to the region just in front of the vitellaria. Due to their small size these glands could be studied in serial cross sections only. They were most prominent just anterior to the testes.

The oral sucker is slightly subterminal and measures 0.11 to 0.15 mm. in diameter. There is no pharynx. The esophagus is short and slightly convoluted with a length about equal to the diameter of the oral sucker. The ceca are long and slender and, as in other members of the family, curve medially to the testes and, hence, lie lateral to the ovary.

The testes are oval organs located at the posterior extremity. They measure 0.28 to 0.56 mm. by 0.15 to 0.28 mm. They are considerably lobed on both the lateral and medial borders although there is some variation in both the pattern and degree of lobation. The vasa efferentia unite anterior to Mehlis' gland to form the vas deferens which proceeds anteriorly in the mid-dorsal line. In the region of the anterior uterine folds the vas deferens enlarges to form the prominent, highly convoluted seminal vesicle. It is a distinctive character of this species that almost the entire seminal vesicle is situated outside the cirrus sac with only a short, anterior portion being enclosed. The relatively small cirrus sac is a straight, slender structure measuring 0.45 to 0.80 mm. in length by 0.035 to 0.056 mm. in width.

The elongate ovary is located between the testes in the posterior region of the body and separated from them by the intestinal ceca. It measures 0.13 to 0.24 mm. by 0.10 to 0.15 mm. The lobation of the ovary varies considerably from specimen to specimen, with an occasional form exhibiting a smoothly oval outline. In most specimens, however, this organ shows slight to moderate lobation anteriorly and one or two prominent and usually unequal projections from the posterior end. Mehlis' gland is a large, irregular structure and lies just anterior to the ovary; it is from 1/3 to 1/2 the size of the ovary. The vitellaria are composed of distinct follicles that are distributed along the lateral aspects of the body from the testes to about the middle. The vitelline ducts arise from the posterior region of the vitellaria and pass medially to the "shell" gland. The uterus exhibits the neat folds characteristic of the family. The number of uterine folds varies from 17 to 22 (av. 19.4) of which 7 to 10 are anterior to the level of the vitellaria. These folds never extend to the cirrus sac, due to the large proportion of the seminal vesicle that is not enclosed in the sac. The weakly muscular metraterm is, in contrast to the other species of the genus, longer than the cirrus sac, and its posterior extremity is in the region of the seminal vesicle. The eggs are 0.018 by 0.011 mm. and are provided with long polar filaments. As has been reported for other species, the male and female pores are separate. In *C. harwoodi* the male pore is located at the level of the middle of the esophagus. The female pore is immediately behind the male pore and in some specimens there appears the suggestion of a genital sinus.

Host: Canada goose: *Branta canadensis* collected at Durham, New Hampshire.

Specimens: U. S. N. M. Helm. Coll. No. 37351, type and paratypes; other paratypes in the collection of W. L. Bullock, Durham, N. H.

Catatropis harwoodi differs from all other species of the genus in having a metraterm that is longer than the cirrus sac and by the large portion of the seminal vesicle that is not enclosed in the sac. These characters are of interest in that previous references to *Catatropis* have usually considered the large cirrus sac with the enclosed seminal vesicle to be of value in identifying the genus. Harwood (1939) has pointed out the weakness of this character due to overlapping with *Notocotylus*. The present material emphasizes this still further. *C. harwoodi* is similar to *C. pricei* and *C. indicus* in the position of the genital pore. It differs from the former in the small size of the cirrus sac and in the failure of the uterus to reach its base. It differs from *C. indicus* on the basis of host and geographic dis-

tribution. (Srivastava's description was not available to the author.) It differs from *C. verrucosa,* the type of the genus, in the size of the cirrus sac, the position of the genital pores, the length of the esophagus, and, as in other forms, the length of the metraterm and the almost entirely unenclosed seminal vesicle.

Genus Paramonostomum Lühe, 1909

The genus *Paramonostomum* was erected by Lühe (1909) to contain *Monostomum alveatum* Mehlis in Creplin. Except for *P. echinum* Harrah (1922) no additional species were described in the genus until the simultaneous description of two American species in 1931: *P. parvum* Stunkard and Dunihue and *P. pseudalveatum* Price. Since 1931 several species have been described from the Orient and South America. The main characteristics of the genus, as indicated by Harwood (1939), appears to be the absence of ventral glands. However, in view of the fact that the glandular pattern of *Catatropis* is often difficult to determine, except in serial section, it seems possible that some of the species of *Paramonostomum* should actually be referred to *Catatropis.* There now appear to be a sufficient number of species without glands to justify this generic character even though Lühe's original concept of the genus was a little too indefinite on this point.

The material on which the present study is based consists of 700 worms taken from the intestine of the Canada goose. About 75 worms were studied in whole mounts. The absence of ventral glands was determined from serial sections of six specimens. For reasons indicated below it was decided to refer these forms to a new species for which the name *Paramonostomum brantae* is proposed.

PARAMONOSTOMUM BRANTAE N. SP.
(fig. 4)

The body is much flattened dorsoventrally and the ventral surface is arched to form a shallow, inverted cup. The specimens under consideration varied from 0.5 to 0.9 mm. in length by 0.3 to 0.5 mm. in width. The anterior extremity is slightly pointed while the posterior end is rounded. Studies of both whole mounts and serial sections failed to produce any evidence of spination of the integument in any portion of the body. Likewise, no evidence for the presence of any type of ventral glands was found.

The oral sucker is slightly subterminal and varied from 0.036 to 0.056 mm. in diameter. A pharynx is lacking. The esophagus is short, broad, and slightly convoluted with a length of 0.04 to 0.05 mm. The ceca are long as in the other representatives of the family and course posteriorly near the sides of the body. In the posterior region they curve medially around the anterior border of the testes and pass between the testes and ovary, ending near the posterior extremity of the body.

The testes are broadly oval organs, measuring 0.10 to 0.16 mm. long by 0.06 to 0.12 mm. wide. They are slightly lobed on both the medial and lateral borders, although the extent of lobation varied from specimen to specimen. In some instances there was a considerable difference in the lobation of the two testes of the same specimen. The vasa efferentia from the testes unite just anterior to Mehlis' gland to form the vas deferens. This latter duct proceeds anteriorly in the middorsal line. The cirrus sac is slightly twisted into an S- or C-shaped curve and is extremely large. It measures 0.12 to 0.25 mm. long by 0.049 to 0.070 mm. wide at its broadest point. The prostatatic portion of the male reproductive tract is prominent and comprises the major portion of the structure in the cirrus sac. A small anterior segment of the seminal vesicle is contained in the posterior quarter of the cirrus sac while the greater portion of the seminal vesicle is free and highly convoluted. It usually bulges anteriorly along the cirrus sac before joining the vas deferens dorsal to the anterior uterine folds. The genital pore is located just posterior to the bifurcation of the gut.

The ovary is a round, slightly lobed organ located between the testes. The posterior margin of the ovary may be slightly anterior, slightly posterior, or on the same level as the posterior end of the testes. The ovary varies in diameter from 0.06 to 0.10 mm. If it is not nearly spherical it is usually broader than long. It usually exhibits, in a given specimen, approximately the same

degree of lobation as the testes. No specimens showed any semblance of a rosette-shaped gonad as described by Kossack (1911) for *P. alveatum*. The large Mehlis' gland is about half the size of the ovary and is located immediately anterior and slightly to the left of it. The vitellaria are composed of discreet, fairly large follicles and extend from the anterior border of the testes to about the middle of the body. They are entirely extracecal. In most specimens the vitelline follicles usually extend slightly more anteriorly on the right side than on the left. (This asymmetry was also noted in some specimens of *P. parvum* that were available to the author). Due to the large size of the cirrus sac the anterior follicles of the vitellaria are usually on a level with this structure even though reaching only slightly anterior to the middle of the body. The vitelline ducts pass medially from the posterior region of the vitellaria and join to form a vitelline reservoir at the anterior end of the Mehlis' gland. The uterus shows the characteristic, regular, closely packed folds of the family. In some specimens these folds are quite distinct whereas in others they are so closely packed as to make counting difficult. Counts of the number of folds of 75 specimens showed a variation of from 8 to 11 with the majority of specimens possessing 9 or 10 (av. 9.9). These folds extend from Mehlis' gland to the base of the cirrus sac which in this form is almost at the middle of the body. The eggs are 0.015 to 0.019 mm. by 0.009 to 0.012 mm. and are provided with long polar filaments.

Host: Canada goose: *Branta canadensis* collected at Durham, New Hampshire.

Specimens: U. S. N. M. Helm. Coll. No. 37352, type and paratypes; other paratypes in the collection of W. L. Bullock, Durham, N. H.

Paramonostomum brantae is a typical representative of the *alveatum* group of the genus as considered by Harwood (1939). It differs from *P. parvum* in having a proportionately larger cirrus sac as well as in the distribution of the vitellaria, and the smaller number of uterine folds. It differs from *P. pseudalveatum* in the size of the cirrus sac, the distribution of the vitellaria, in the larger number of uterine folds, and in the position of the genital pore. It most closely resembles *P. alveatum,* the type of the genus, but lacks the rosette-shaped lobation of the ovary as indicated by Kossack (1911) and possesses a larger cirrus sac than indicated by any of the figures of this long known but enigmatic form.

DISCUSSION

The separation of *P. brantae* from *P. alveatum* proved difficult due to the multiplicity of conflicting descriptions of the European form. Creplin (1846) simply gave the host record (*Anas berniclae*) of *M. alveatum* and attributed the species to Mehlis. Wedl (1858) described *M. verrucosum* (ex *Fulica atra*) which Kossack (1911) assigned, with some misgivings, to *M. alveatum*. Reference to Wedl's paper by the present author led to still further uncertainty regarding the identity of Wedl's form. The trematode figured and described by Wedl does not even appear to belong to the subfamily NOTOCOTYLINAE. The ceca are outside of the testes; there is no esophagus; and the vitellaria extend from the genital pore to the extreme posterior end. It is, therefore, extremely doubtful that *M. verrucosum* Wedl 1858 should be considered as a synonym of *M. alveatum* Mehlis in Creplin 1846.

Recent workers have given more consistent descriptions of this European form. Monticelli (1892) studied and described the then dried-up original Mehlis' material. Mühling (1898) based his description on "millions" of small yellowish worms from *Fuligula marila*. The latter author considered his specimens to be identical with the original Mehlis' material. Lühe (1909) redescribed the species and erected the genus *Paramonostomum* on the basis of the apparent absence of ventral glands. Kossack (1911) gave a rather complete description in his lengthy consideration of the monostomes. Harrah (1922) claimed to present the description of Lühe (1909) while Dawes (1946) appeared to rely on the descriptions of both Lühe

(1909) and Kossack (1911). The descriptions of these various workers, unfortunately, exhibited discrepancies not only concerning the relative importance of certain morphological features, but the very presence or absence of these features in the species.

The cuticle was considered as "unbestachelt (?)" by Lühe. According to Kossack and Harrah, however, it had "fine spines" and "heavy set spines" respectively. Dawes considered the integument to be "apparently smooth". Although all of these workers agreed in their text or in their figures or both that the testes were irregular in outline, the nature of the lobation of the ovary was more uncertain. Mühling and Lühe figured the ovary as moderately lobed but Kossack considered a highly lobed ovary with a rosette pattern to be an important characteristic of the species.

The vitellaria were described as extending anteriorly to the middle of the body or as taking up the middle body third. However, all figures, except that of Monticelli, showed the vitellaria extending to a short distance anterior to the middle of the body. Monticelli's figure was not clear but indicated the vitellaria as extending to the end of the cirrus sac. It may be important in this regard to remember that Monticelli's material was in poor condition and, therefore, not too reliable. The cirrus sac in all cases appeared to extend back to the end of the anterior body third.

TABLE 1.—*The ratios of the distribution of the vitellaria and the posterior limit of the cirrus sac as related to total body length in the 4 species of* Paramonostomum *under consideration.*

	P. parvum	*P. pseudalveatum*	*P. alveatum*	*P. brantae*
Anterior extent of vitellaria / Total body length	0.34	0.28	0.48	0.42–0.50 av. 0.47
Posterior limit of cirrus sac / Total body length	0.34	0.28	0.33	0.41–0.48 av. 0.46

The nature of the seminal vesicle is also a character that is not consistently described. Lühe considered this structure to be "stark gewunden". Dawes took issue with Lühe and described it as "not convoluted". The basis for his disagreement was not given. Kossack referred to the portion contained *in the cirrus pouch* as "gar nicht gewunden" but did not mention the degree of convolution of the greater portion of the seminal vesicle which he said lay outside the sac. It is possible that Lühe and Dawes were referring to two different portions of the same structure.

These disagreements in the literature prompted a more detailed study of the species characters in *Paramonostomum*. Measurements were taken of the number of uterine folds and the distance from the anterior end of the body to the posterior end of the cirrus sac and the anterior limit of the vitellaria. These measurements were made on 75 specimens of *P. brantae,* 3 specimens of *P. parvum* (kindly sent to the author by H. W. Stunkard, who suggested the study of the uterine folds), 3 specimens of *P. parvum* and one of *P. pseudalveatum* from the U. S. National Museum. From these measurements the ratios indicated in Table 1 were calculated. The ratios for *P. alveatum* were determined from the figures of Mühling (1898), Lühe (1909), and Kossack (1911).

These data indicate that the relationship of the forward distribution of the vitellaria to the posterior end of the cirrus sac is a significant specific character, but only

if the total body distribution of the vitellaria is also considered. The number of uterine folds is probably of considerable importance but, due to difficulties in making accurate counts, should be used only in conjunction with other characters. Both of these criteria are subject to some variation and further studies on other populations may invalidate their use. Herber (1942) in his study of *Quinqueserialis quinqueserialis* and *Notocotylus stagnicolae* considered the following to be useful specific characters for the NOTOCOTYLINAE: "(1) the number of ventral glands in each row, (2) the anterior extent of the vitellaria, (3) the lateral lobation of the testes, (4) the body and tail size of cercariae, and (5) the course of the excretory tubules of the cercariae". The last two characters are useful only if the life cycle can be successfully duplicated. The nature of the ventral glands is useful only in those genera which possess them, and consequently, of no value in *Paramonostomum.* The distribution of the vitellaria appears to be of value in *Paramonostomum* if considered in relation to the cirrus sac and total body length. Further study of this character is needed in *Catatropis.* The degree of lobation of the testes (medially or laterally) seems to be of little value in either *Catatropis* or *Paramonostomum* as evidenced by the populations of the two species reported in the present study. The degree of lobation of the ovary appears to be equally unreliable. In spite of Kossack's emphasis on the rosette pattern of *P. alveatum* other workers show only moderate lobation.

Apparently much of the confusion in the literature as to the significant specific characters of *P. alveatum* is due to an overemphasis on variable characters. Thus, the rosette-shaped ovary of Kossack and the C-shaped anterior fold of Kossack and Dawes are characters that now appear to be highly variable in *Paramonostomum.* The nature of the seminal vesicle (convoluted or straight) and the spination of the anterior region of the body appear to be misinterpreted and should, therefore, be reinvestigated before their reliability as specific characters can be ascertained. The most reliable characters for the separation of the known species of the genus appear to be the relation of the distribution of the vitellaria to the size of the cirrus sac, the number of folds of the uterus, and the position of the genital pore.

SUMMARY

1. *Catatropis harwoodi,* n. sp. and *Paramonostomum brantae,* n. sp. are described from a Canada goose massively infected with both species.

2. The relation of *C. harwoodi* and *P. brantae* to other species of the genera is indicated.

3. From these studies it appears that the relation of the posterior end of the cirrus sac and the anterior distribution of vitellaria in terms of total body distribution of the vitellaria is of importance for differentiating species of *Paramonostomum.* Although variable, the number of uterine folds also appears to be of some value.

4. Using these characters it is possible to differentiate the European and North American species of *Paramonostomum.*

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Explanation of Plate.

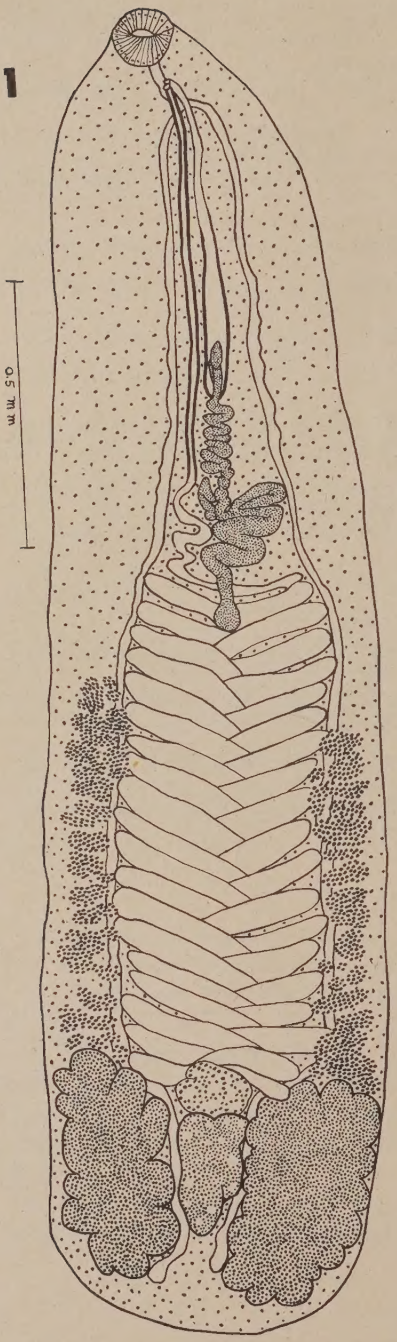
Fig. 1. Whole mount of *Catatropis harwoodi,* n. sp. Note small cirrus sac and prominent, convoluted seminal vesicle outside of the sac.

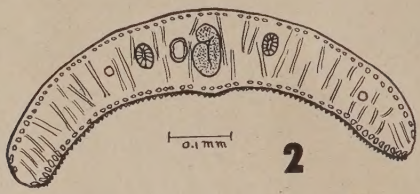
Fig. 2. Cross section of *C. harwoodi* at level of anterior loops of the seminal vesicle. Note metraterm to left of seminal vesicle as well as ventral spines and anterior end of midventral glandular ridge.

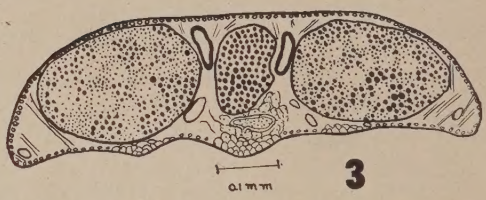
Fig. 3. Cross section of *C. harwoodi* at level of gonads. Note ventral glands on either side of glandular ridge.

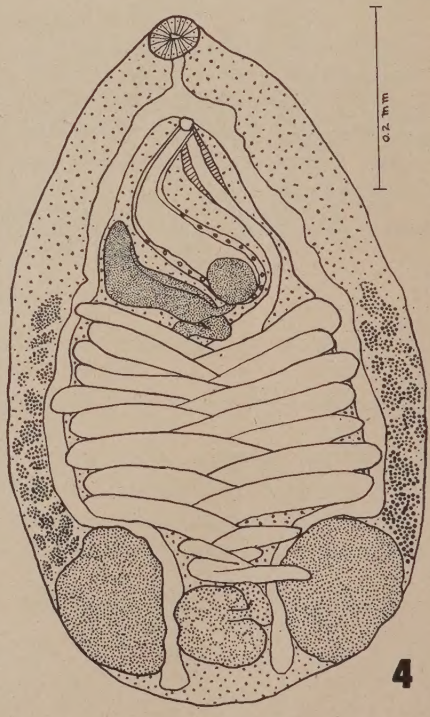
Fig. 4. Whole mount of *Paramonostomum brantae,* n. sp. in which ratio of anterior distribution of vitellaria to total body length is 0.43.

PLATE I









SOME NEW OXYURID NEMATODES FROM SOUTHERN CALIFORNIA

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Many investigations have been made of reptiles of Southern California. The great variety of terrestrial reptiles in this area has stimulated such studies. On the other hand, it is surprising to find that little is known of the helminth parasites of cold-blooded land vertebrates in the arid regions of the Southwestern United States.

The authors have initiated a systematic study of the oxyurid nematodes occurring in reptiles in Southern California. In this and subsequent portions of this study a number of new species will be described, and pertinent notes on the distribution and biology of these forms will be presented.

The Genus *Thelandros* Wedl, 1862

The genus *Thelandros* consists of twenty-three described species. Of these, twenty-two species have been described from reptiles and one from an amphibian. Walton (1941) reviewed the geographical and host distribution of the genus and pointed out that the majority of the known species are from North African hosts. A few species have been described from Asian and South American hosts and one from Australia; at present four species are known, though not completely described, from North America. Walton (1941) described the female of an unnamed *Thelandros* from the California legless lizards, *Anniella pulchra* and *A. nigra.* Walton surmised that the genus is more widely distributed than previous studies would indicate, which opinion is supported by the data of the present paper.

Lucker (1951) has recently described, in abstract, three species of *Thelandros* from the night lizard, *Xantusia riversiana reticulata* Smith, taken on San Clemente Island, California. As far as the present authors can ascertain from Lucker's very brief descriptions, the three species from *X. riversiana reticulata* are not specifically identical with the species of *Thelandros* described herein from *Xantusia vigilis, X. henshawi,* and *X. riversiana riversiana.*

Thelandros californiensis n. sp. (Figs. 1–5)

Study of oxyurids collected from the night lizards, *Xantusia vigilis* and *X. henshawi* indicated that these might be specifically identical with the *Thelandros* sp. described by Walton (1941) from *Anniella pulchra* and *A. nigra.* Direct comparison of our material from xantusiids with specimens kindly sent to us by Dr. Walton has not substantiated this opinion.

Specific diagnosis: Female: 7.0 to 10.0 mm. long; 0.60 to 0.65 mm. wide at the vulva. Esophagus 0.75 to 1.00 mm. long; bulb 0.20 to 0.28 mm. in diameter. Tail 0.380 to 0.425 mm. long; anus-tail distance 0.720 to 0.765 mm. Vulva median. Ovarian coils prebulbar and circumesophageal. Eggs 0.048 to 0.052 mm. by 0.090 to 0.110 mm. with single subpolar plug and initial segmentation at time of oviposition. Excretory pore postbulbar, about 0.160 mm. posterior to esophageal-intestinal junction.

Male: 1.8 to 2.5 mm. long; maximum width 0.099 to 0.165 mm. Esophagus 0.214 to 0.280 mm. long. Lateral and caudal alae lacking. Pointed, dorso-posteriorly projecting process 0.080 to 0.105 mm. long; 1 preanal, 1 lateral adanal, and 2 postanal pairs of mammillary papillae

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present. Posterior pair of papillae situated on dorso-posterior projection. Single spicule 0.053 to 0.076 mm. long, grooved proximally.

Hosts: *Xantusia vigilis* Baird (type) and *X. henshawi* Stejneger.

Location: Caecum and large intestine.

Locality: Palmdale, Los Angeles County, California.

Holotype male and allotype female: U. S. Natl. Mus. Helm. Coll. No. 37348.

Paratypes: Department of Zoology, University of California, Los Angeles.

The adult of *T. californiensis* is a transparent, transversely straited worm with no trace of the spination so conspicuous in the pre-adult. Nor is there any furry covering as has been reported in *Oxyuris* sp. of Thapar (1925), which is a *Thelandros* sp., and in *Thelandros rotundus* Malan, 1939. The transverse rings of striation are 20 to 40 micra apart in *T. californiensis*. The lateral areas of the worm are marked, particularly in living specimens, by large elliptical cells with spherical nuclei. Lateral cuticular ridges are sometimes visible, but lateral alae are not present. The mouth is surrounded by 3 bilobed lips; the buccal cavity is weakly developed.

In the female, the vulva has slightly projecting lips and leads into a fairly heavily muscularized ovejector which in turn connects with the two converging uteri. Each uterus, in the mature worm, is filled with the brown eggs. The uterine coils descend almost to the level of the anus posteriorly, and, anteriorly, the uterine coils ascend into the first quarter of the worm. The uteri are much folded and make up the greatest bulk of the body contents. The grayish ovaries loop 3 to 5 times around the corpus of the esophagus, anterior to the bulb. The eggs are oval in shape, but exhibit a typically flattened side in lateral view. At the time of oviposition the brown egg shell shows a fine regular stippling. At this time the eggs are in the 4-, 8- or 16-celled stage.

T. californiensis resembles *Thelandros* sp. of Walton (1941) in the circumesophageal prebulbar ovarian coils. However, *T. californiensis* is a much larger form than the worm described by Walton. In addition, study of the eggs *T. californiensis* reveals that they consistently differ from those of Walton's *Thelandros* from *Anniella*. In the material described by Walton the eggs are narrower and thicker-shelled than those of *T. californiensis*. In addition, the polar plug in the egg of the worm from *Anniella* spp. is terminal while the polar plug in the egg of *T. californiensis* is always subterminal. It is evident that the *Thelandros* described by Walton is not specifically identical with *T. californiensis*. Thus, it is proposed that the species from *Anniella* discovered by Walton be named *Thelandros waltoni* n. sp. An effort is being made to obtain additional material from *Anniella* in order to study the morphology of the male of *T. waltoni*.

In possessing circumesophageal ovarian coils, *T. californiensis* shows affinities with *T. maplestoni* (Chatterji), *T. micipsae* Seurat, *T. rotundus* Malan, *T. sceleratus* Travassos, and *T. seurati* Sandground. *T. californiensis* differs from *T. maplestoni* in possessing mammillary, somewhat laterally located posterior papillae in the male rather than the small adanal papillae of *T. maplestoni*. The present species differs from *T. micipsae, T. rotundus,* and *T. sceleratus* in lacking the caudal alae reported for these species. *T. seurati* is unlike *T. californiensis* in having a fringed anterior cloacal border and in other features of the caudal end of the male.

Both *Xantusia vigilis* and *X. henshawi* showed a rather high incidence of infection with *Thelandros californiensis*. Of 100 *X. vigilis* examined 20% harbored

this worm; of 20 *X. henshawi* examined 40% were infected. In all cases female worms far outnumber males. Infected animals contained up to 9 adult worms although, in some cases, there were additional immature worms.

Of great interest was the frequent observation of 4th stage (?) larvae of *T. californiensis*. These young worms differ markedly from the adults in possessing hooklike spines over the entire body. The spines on the posterior part of the body are somewhat larger than the more anterior ones. We were at first somewhat inclined to doubt that these singular larvae belonged to the same species as the adult *Thelandros* found in these xantusiids. However, on two occasions we have observed exuviation, and the freshly-emerged larvae were indistinguishable from immature *Thelandros* from the same host. Figure 3 shows one of these larvae in the process of exuviation. A search of the literature reveals that Seurat (1917) observed similar spiny larvae in the gecko, *Tarentola mauritanica*. These were referred by Seurat to *Thelandros echinatus* from the same host. The present findings may support Seurat's conclusions. Further study may show that the spines are a general character of the pre-adult form in this genus.

In the larva of *T. californiensis* no reproductive anlagen can be discerned; the digestive tract is the prominent internal structure. It was noted that these spiny larvae exhibit peculiar contractions, somewhat peristaltic in nature. Such movement, with the aid of the backward-curved spines, may result in progression. Such movements are not observed in the adults.

Thelandros bicaudatus n. sp. (Figs. 6–9)

This species was collected from fifteen of nineteen *Xantusia riversiana riversiana* taken at San Nicolas Island, California.

Specific diagnosis: Female: 3.24 to 4.22 mm. long; 0.255 to 0.456 mm. wide at the vulva. Esophagus 0.690 to 0.984 mm. long; bulb 0.092 to 0.135 mm. in diameter. Vulva slightly posterior to middle of body. Tail 0.198 to 0.240 mm. long. Lateral lines usually visible. Excretory pore postbulbar, about 1.0 mm. from anterior end. Reproductive organs confined to middle third of body. Eggs 0.049 to 0.060 mm. by 0.092 to 0.120 mm. with small terminal operculum. Embryos in morula or later stage at oviposition.

Male: 2.010 to 3.075 mm. long; maximum width 0.151 to 0.225 mm. Esophagus 0.491 to 0.645 mm. long; bulb 0.075 to 0.097 mm. in diameter. Posterior end truncate, but terminated by conical process with inflated tip. Dorsal caudal projection 0.085 to 0.102 mm. long. One median and one lateral pair of sessile preanal papillae present; one pair of small, sessile postanal papillae at midpoint of dorsal projection. Spicule slender, 0.165 to 0.180 mm. long, rounded distally. Lateral and caudal alae lacking. Excretory pore about 0.375 mm. posterior to esophageal-intestinal junction.

Host: Xantusia riversiana riversiana Cope.
Location: Caecum.
Locality: San Nicolas Island, California.
Holotype male and allotype female: U. S. Natl. Mus. Helm. Coll. No. 37349.
Paratypes: Department of Zoology, University of California, Los Angeles.

In the postesophageal reproductive organs and in lacking lateral and caudal alae, *T. bicaudatus* resembles *T. alatus* Wedl, *T. micruris* Rauther, and *T. sahariensis* Baylis. The male of *T. bicaudatus* differs from *T. alatus* in the postanal conical process and in the absence of a fringed cloacal border. Both *T. micruris* and *T. sahariensis* have the cauda enveloped in a prepuce-like structure. This has not been observed in *T. bicaudatus*.

Thelandros minutus n. sp. (Figs. 10–12)

This species was collected from the caudate amphibian, *Batrachoseps attenuatus attenuatus*, taken in a public park in Los Angeles, California. Appreciation is expressed to Mr. David

Doran of this department, who collected these hosts. Of thirty-seven hosts examined eight harbored this oxyurid.

Specific Diagnosis: Female: 4.08 to 4.68 mm. long; width at the vulva 0.384 to 0.600 mm. Esophagus 0.388 to 0.600 mm. long; isthmus of bulb about 0.017 mm. long; bulb 0.079 to 0.092 mm. long by 0.083 to 0.102 mm. wide. Nerve ring about 0.070 mm. from anterior end. Vulva median. Reproductive organs postbulbar. Anus-tail distance 0.336 to 0.480 mm. Eggs 0.049 to 0.062 mm. by 0.112 to 0.119 mm. with single subpolar plug and initial segmentation at time of oviposition. Excretory pore postbulbar.

Male: 1.49 to 1.63 mm. long; maximum width 0.089 to 0.100 mm. Esophagus 0.204 to 0.231 mm. long; bulb 0.049 to 0.059 mm. in diameter. Posterior end truncate. Dorsal caudal projection 0.034 to 0.041 mm. long. One pair of preanal and two pairs of postanal papillae present. Posterior pair of papillae on inflated basal portion of dorsal caudal projection. Slender spicule 0.040 to 0.056 mm. long. Lateral and caudal alae lacking.

Host: Batrachoseps attenuatus attenuatus.
Location: Large intestine
Locality: Los Angeles, California
Holotype male and allotype female: U. S. Natl. Mus. Helm. Coll. No. 37347.
Paratypes: Department of Zoology, University of California, Los Angeles.

T. minutus most resembles *T. alatus* Wedl and *T. bicaudatus* (this paper). It differs from *T. alatus* in size and and lacking the serrate anterior cloacal border described in the male of that species. No postanal conical process, such as in *T. bicaudatus,* is present in the male of *T. minutus.*

The Genus *Pseudoalaeuris* Walton, 1942

This genus was erected by Walton (1942) to include certain syphacinid species in which the males have caudal alae, but in which both sexes lack any trace of lateral alae. In *Pseudoalaeris* Walton included several species which previous workers had referred to *Alacuris* or *Tachygonetria.* In the same paper Walton described three new species of *Pseudoalaeuris.* Of the fourteen described species of this genus seven are known from Galapagos; the remaining species occur in Afghanistan, South Africa, and South and Central America. None have previously been reported from reptiles in the U. S.

Pseudoalaeuris waltoni n. sp. (Figs. 13–16)

Oxyurids of this species were collected from fourteen of nineteen night lizards, *Xantusia riversiana riversiana* Cope. taken at San Nicolas Island, California.

Specific diagnosis: Female: 2.3 to 2.6 mm. long; 0.250 to 0.280 mm. wide at the vulva. Esophagus, including bulb, 0.80 to 0.90 mm. long; bulb 0.120 to 0.138 mm. in diameter. Vulva slightly posterior to mid-body with prominent prevulvar flap; none of reproductive structures extend to bulbar region. Excretory pore postbulbar. Conical tail. No specialized cuticular modifications. Eggs 0.056 to 0.066 by 0.095 to 0.128 mm., relatively thick-walled with terminal thinned opercular area and initial segmentation at time of oviposition.

Male: 2.625 to 2.925 mm. long; 0.16 to 0.25 mm. wide; body anteriorly drawn out, posterior extremity cut away ventrally; dorso-posteriorly projecting pointed tail with prominent bilobed caudal alae; one median preanal fused pair of processes, one lateral pair of perianal finger-like processes, and one smaller sessile pair of caudal papillae at level of injunction of alae and tail spike; single acicular spicule 0.150 to 0.195 mm. long; accessory piece Y-shaped, 0.038 to 0.043 mm. long.

Host: Xantusia riversiana riversiana Cope.
Location: Caecum.
Locality: San Nicolas Island, California.
Holotype male and allotype female: U. S. Natl. Mus. Helm. Coll. No. 37350.
Paratypes: Department of Zoology, University of California, Los Angeles.

P. waltoni resembles *P. conolophi* (Cuckler, 1938), *P. inflatocervix* (Akhtar, 1937) and *P. macroptera* Walton, 1942, in having a postbulbar excretory pore. *P. waltoni* differs from *P. conolophi* in the size of the spicule and in the arrangement of the caudal papillae. Unlike *P. inflatocervix, P. waltoni* does not have a cuticular

cervical collar. The males of *P. macroptera* and *P. waltoni* differ in the arrangement of papillae and in the shape of the caudal alae.

SUMMARY

Descriptions are given of *Thelandros californiensis* n. sp. from *Xantusia vigilis* and *X. henshawi, Thelandros bicaudatus* n. sp. from *Xantusia riversiana riversiana; Thelandros minutus* n. sp. from *Batrachoseps attenuatus attenuatus,* and *Pseudoalaeuris waltoni* n. sp. from *Xantusia riversiana riversiana.* The *Thelandros* sp. described by Walton (1941) is named *Thelandros waltoni* n. sp.

ADDENDUM

After the present paper was accepted for publication, J. T. Lucker published the results of a restudy of *Thelandros alatus* Wedl (Jour. Parasitol. 38: 69–75). Lucker presented evidence that *T. micruris* Rauther, *T. sahariensis* Baylis, and *T. avis* Maplestone are synonyms of *T. alatus. T. bicaudatus,* described in the present paper, differs from *T. alatus,* most obviously, in having a spicule about twice as long as that of the latter species.

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PLATE I.

Thelandros californiensis

FIG. 1. Female, entire. FIG. 2. Spiny infective larva. FIG. 3. Exuviating larva; drawn from living specimen. FIG. 4. Egg. FIG. 5. Caudal end of male, lateral view.

Thelandros bicaudatus

FIG. 6. Caudal end of male, ventral view. FIG. 7. Caudal end of male, lateral view. FIG. 8. Egg. FIG. 9. Caudal end of female, lateral view.

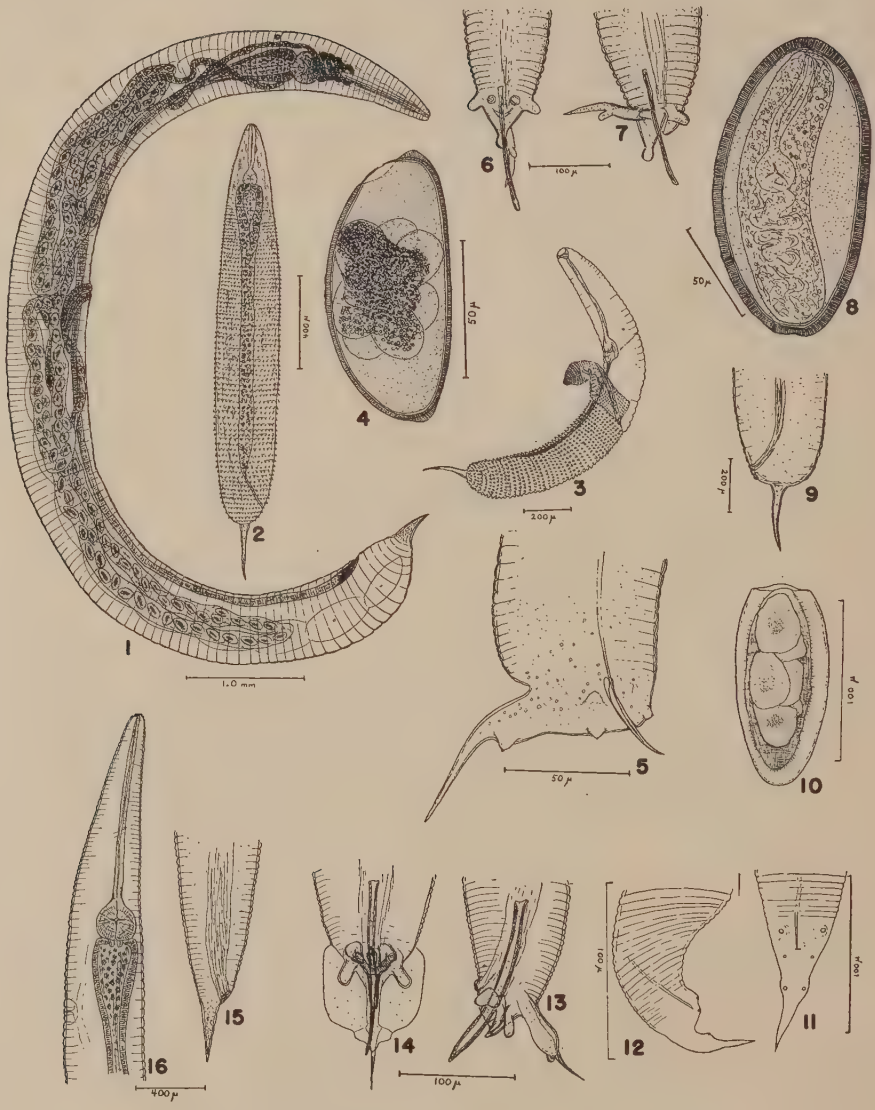
Thelandros minutus

FIG. 10. Egg. FIG. 11. Caudal end of male, ventral view. FIG. 12. Caudal end of male, lateral view.

Pseudoalaeuris waltoni

FIG. 13. Caudal end of male, lateral view. FIG. 14. Caudal end of male, ventral view. FIG. 15. Caudal end of female, lateral view. FIG. 16. Anterior end of female, lateral view.

PLATE I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TWO NEW NASAL MITES FROM COLUMBIFORM BIRDS

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In a survey of the respiratory parasites of columbiform birds performed at Texas Technological College, Lubbock, Texas, two new species of nasal mites were discovered: *Neonyssus zenaidurae,* from the mourning dove, and *Speleognathus striatus,* from the domestic pigeon. Mexican ground doves were also examined, but only one of sixteen birds was parasitized. Other species of columbiform birds were not available for study.

Family SPELEOGNATHIDAE (Trombidiformes)

Speleognathus striatus, new species (Pl. I)

Female: Body length, excluding gnathosoma, 423–537 μ; average, 466 μ. Body width, 295–448 μ; average, 343 μ. Color, white. A pair of eyes is present.

Dorsum: (figs. 5, 14). No plates present. Eight pairs of plumose setae and one pair of attenuate setae as illustrated. A single pair of eyes present; each eye located above Leg I, accompanying the attenuate seta. A clear lens is visible. The entire dorsum is covered with very fine, short striations which, under high magnification, give the body a ridged appearance (see figs. 9, 12).

Venter: (figs. 1, 17). No plates present. Twelve pairs of ventral plumose setae as illustrated. No attenuate setae. Genital opening a longitudinal slit behind coxa IV; flanked by five pairs of setae (fig. 17). Anus located posteriorly; sometimes terminal. Striations similar to those of the dorsum.

Legs: (figs. 2, 3, 4, 6, 7, 15, 16). Slender; the segments somewhat rounded, and slightly constricted at their unions so that the entire leg has the appearance of a string of beads. Tarsi swollen, provided with claws (figs. 2, 3, 4, 6, 7). Tufted empodium present on each tarsus. Several plumose setae located distally on all tarsi; other setae as illustrated. All legs provided with an internal sclerotized network (figs. 15, 16). Striations similar to those of the body.

Gnathosoma: (figs. 8, 9, 11–13). Located ventrally; partially visible dorsally. Palps three-segmented; two tufted setae and one bulbous seta on the ventral side of the palpal tarsus (fig. 9). On the dorsal side, the palps bear two peculiar setae, one on the palpal tarsus and one on the middle palpal segment (fig. 12). These structures appear to be cleft, but the two halves were never found to be spread. Their small size and their transparency make them difficult to observe. The chelicerae are the most dorsal structures; without teeth, but with a sclerotized spine on the ventral surface (fig. 8). The beak is conical, and contains several unrecognized structures. Two small setae present on the ventral surface of the beak. Striations raised, similar to those of the body.

Male: Unknown.

Immature Forms: (fig. 10). Although neither nymphs nor larvae were found, several females contained developing larvae (fig. 10). The larvae were all very large in relation to the bodies of the females containing them.

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Host and Locality: Taken from *Columba livia domestica,* the domestic pigeon, in Garza Co. (Travis R. Everett collector) and in Lubbock Co., Texas.

Types: The holotype female and three paratypes are deposited in the U. S. National Museum No. 2,004.

This species is easily distinguished from the type species, *Speleognathus australis* Womersley (1936), which has one-segmented palps according to the description. The pattern of setae is slightly different; also, *S. australis* has a pair of attenuate setae just anterior to the genital opening whereas *S. striatus* has no attenuate setae on the venter.

Speleognathus striatus may be distinguished from the remaining member of the genus, *S. sturni* Boyd (1948), by the absence of eyes in the latter.

The type species of the family Speleognathidae, *Speleognathus australis,* was found in moss and has never been reported from a bird. In 1948, Boyd placed in this genus a nasal mite (*S. sturni*) from the starling. The many similarities of the two mites justified this action. Dr. Wormersley has suggested (private correspondence) that the type species, *S. australis,* may be a nasal mite and that drinking water may be the vehicle of transmission. The author is in complete agreement. *Speleognathus striatus* has a hydrophobic cuticle, enabling the mite to float on the surface of water, and also is able to run quite rapidly.

Neonyssus melloi Castro (1948) and *N. columbae* Crossley (1950) were also recovered from pigeons in this survey. Sixty pigeons were examined; nasal mites were collected from thirty-one (57 per cent) of the birds. *Speleognathus striatus* was found in 15 percent of the pigeons, *N. melloi* in 25 percent, and *N. columbae* in 20 percent. On two occasions, *S. striatus* and *N. columbae* were found in the same pigeon; *S. striatus* was never found with *N. melloi. Neonyssus melloi* and *N. columbae* were not found together.

Family RHINONYSSIDAE (Parasitiformes)

Neonyssus zenaidurae, new species (Pl. II)

An oval mite, with stout legs and reduced setation. Gnathosoma visible from above, and terminal in position.

Female: (figs. 1, 3–5, 7–9, 11–13). Body length, excluding gnathosoma, 512–634 μ; average, 601 μ. Body width, 384–486 μ; average, 448 μ.

Venter: (figs. 4, 7, 8). Sternal plate absent. Three pairs of sternal setae; the first and second pair followed by sternal pores, as illustrated. Genital plate (fig. 4) oblong; slightly sclerotized, opening anteriorly; flanked by one pair of setae anteriorly and two pairs posteriorly. Anal plate (fig. 7) oval, elongate posteriorly, with cribrum extending to dorsum. One pair of setae lateral to anal pore; one seta on posterior part of plate. Twelve pairs of attenuate, well-developed setae present on posterior end of abdomen. Venter striated as illustrated.

Dorsum: (figs. 5, 9). Two plates cover most of the dorsum. Borders of the plates may be irregular, especially the anterior border of the opistosomal plate. Both plates heavily sclerotized and sculptured. Opistosomal plate fan-shaped posteriorly, and slightly wider than the podosomal plate in this region. Stigmas (fig. 9) located lateral to the posterior border of the podosomic plate; peritreme short, with annular thickenings. Sub-cutaneous plates, which may represent peritrematalia, extend posteriorly from the stigmas. Six small setae located on the posterior portion of

the dorsum, immediately behind the opistosomal plate. Dorsum striated as illustrated.

Legs: (fig. 3). Stout; setae reduced in number and size. Sclerotized areas regular in shape. Dorsal setae of two types: (1) attenuate, with a very thin basal portion, and (2) stumpy, with the basal portion thick. Ventral setae mostly attenuate, several stumpy setae present on legs I and II. Strong attenuate setae found on the tarsi of all legs and on coxae II and III. Claws stout, well-developed.

Gnathosoma: (figs. 1, 11–13). Palps five-segmented. Palpal tarsus unusually prominent, projecting slightly from the tibia. Two unusually long setae present apically on palp (fig. 12). A small seta with expanded distal portion at the base of the tarsus. Several small attenuate setae on palpal tarsus and tibia; other palpal setae stout. Hypostome without capitular groove; two ventrolateral stout setae on the base. Six stout setae on hypostomal processes. Labrum indistinct. Chela shear-like, well-developed (fig. 1); a tooth may be present near the base of the fixed digit. Epistome projecting forward to the first palpal segment; very membranous at tip.

Male: (figs. 2, 6, 10). Very similar to female, slightly smaller. Length, 410–538 μ; average 486 μ. Width 332–397 μ; average 358 μ.

Movable digit of male chela reduced in size; spermatophore carrier as long as fixed digit (fig. 2). A distinct tooth on the fixed digit. Ventral plate present (fig. 6), well-sclerotized, and surrounded by a clear area. One pair of genital setae slightly below the genital opening. First pair of sternal pores located immediately posterior to the genital setae; second pair accompanies the first pair of sternal setae.

Immature Forms: Six immature forms were collected. All of these proved to be nymphs, four of which were in the process of moulting; the other two were inadequate for illustrations. It is the author's intention to publish illustrations at a later date.

Apparently two nymphal stages were present. These may be separated by the chela: One, probably a protonymph, has a poorly developed chela; the other, probably a deutonymph, has a chela similar to that of the female. Otherwise, the two stages appear to be identical.

Abdomen with four pairs of short, attenuate setae, plus the three anal setae. In addition, two terminal abdominal spines. Several spots of sclerotization on the dorsum, but no organized plates. Legs short and stout. Otherwise similar to the adult.

Types: The holotype female, two paratype females, and two paratype males are deposited in the U. S. National Museum No. 2,005.

Type Host: mourning dove, *Zenaidura macroura*. Also collected from the Mexican ground dove, *Columbigallina passerina*.

Type Locality: Dickens Co., Texas, R. W. Strandtmann collector. Also collected in Lubbock Co. and in Caldwell Co., Texas. Two females were collected in Grady County, Georgia, by Roy Komarek and H. B. Morlan, U. S. P. H. S.

The fan-shaped opistosomic plate and the two long setae on the tip of the palp readily distinguish this mite from the other members of the genus. Other characters valuable in diagnosis include the long peritrematalia, the attenuate setae on the dorsal side of the legs, the sternal pores, and the sclerotized ventral plate of the male. These latter characters suggest a close relationship between *Neonyssus zenaidurae* and the ectoparasitic mites.

Neonyssus zenaidurae was the only nasal mite found in the mourning dove. Nineteen mourning doves were examined; ten were found to be infested. Mexican ground doves were taken in Kleberg Co., Texas, by Larry F. Cavazos. Sixteen were examined and only one contained mites, two females of *N. zenaidurae*. This is probably an accidental record.

Castro and Pereira (1949) divided the genus *Neonyssus* into two sub-genera: (1) *N.* (*Neonyssus*), with two large dorsal shields, one podosomic and the other opistosomic; and (2) *N.* (*Ptilonyssoides*), with three dorsal plates; one large podosomic plate and two opistosomic, the latter two consisting of one anterior median plate and one posterior median plate. *Neonyssus zenaidurae* belongs to the former sub-genus.

ACKNOWLEDGMENTS

The author is sincerely grateful to the persons who have contributed their aid to this study. Special acknowledgment is due to Dr. R. W. Strandtmann of Texas Technological College, Lubbock, Texas; his excellent advice and criticisms were of invaluable assistance.

SUMMARY

Two new species of mites are described: *Speleognathus striatus* (Trombidiformes: Speleognathidae) from the domestic pigeon (*Columba livia domestica*), and *Neonyssus zenaidurae* (Parasitiformes: Rhinonyssidae) from the mourning dove (*Zenaidura macroura*). *Speleognathus striatus* possesses eyes, three-segmented palps, and lacks ventral attenuate setae; fifty-two specimens were examined for the description. *Neonyssus zenaidurae* may be distinguished by its fan-shaped opistosomal plate and its two long palpal setae; thirty-eight specimens were examined.

Neonyssus zenaidurae is also recorded from the Mexican ground dove, *Columbigallina passerina*. Only one of sixteen birds was parasitized.

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EXPLANATION OF PLATE I

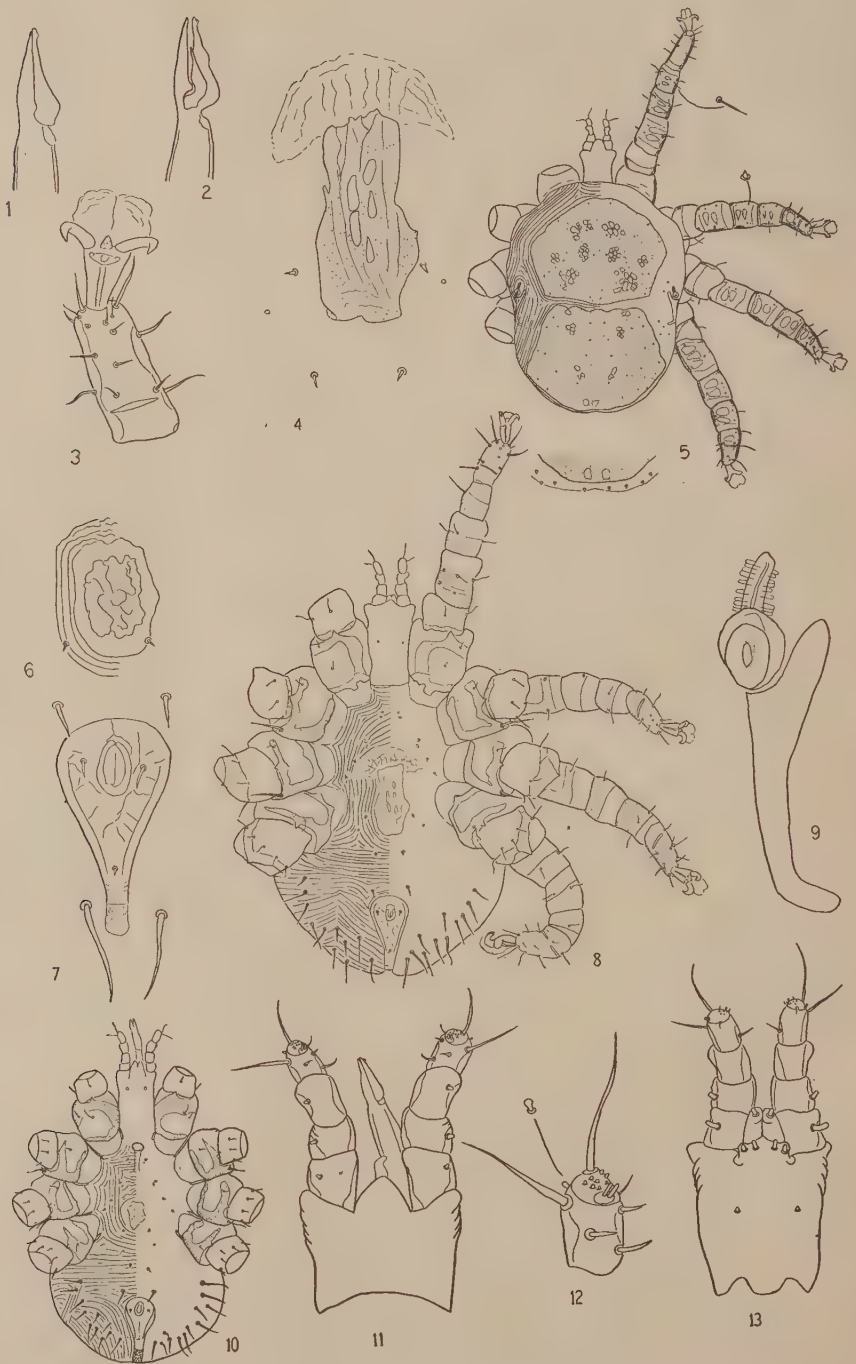
Speleognathus striatus: Female. Fig. 1, ventral view; fig. 2, tarsus I, dorsal view; fig. 3, tarsus I, ventral view; fig. 4, tarsus IV, side view; fig. 5, eye; fig. 6, tarsus II, dorsal view; fig. 7, tarsus II, ventral view; fig. 8, spine of chelicera; fig. 9, palpal tarsus, ventral view; fig. 10, female with larva; fig. 11, gnathosoma, dorsal view; fig. 12, palp, dorsal view; fig. 13, gnathosoma, ventral view; fig. 14, dorsal view of body; fig. 15, leg I, dorsal view; fig. 16, leg I, ventral view; fig. 17, genital opening and anus.

EXPLANATION OF PLATE II

Neonyssus zenaidurae: Fig. 1, chela of female; fig. 2, chela of male; fig. 3, tarsus I of female, ventral view; fig. 4, genital plate of female; fig. 5, dorsal view of female; fig. 6, ventral plate of male; fig. 7, anal plate of female; fig. 8, ventral view of female; fig. 9, stigma and peritreme of female; fig. 10, ventral view of male; fig. 11, gnathosoma of female, dorsal view; fig. 12, palpal tarsus of female, dorsal view; fig. 13, gnathosoma of female, ventral view.

PLATE I

PLATE II



BIONOMICS OF TWO TREMATODE PARASITES OF NEW ZEALAND EELS

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The freshwater eels of New Zealand carry two trematodes in the intestine. They have been described as *Telogaster opisthorchis* Macfarlane (1945) and *Stegodexamene anguillae* Marfarlane (1951). The larval stages of the parasites pass from the gastropod, *Potamopyrgus antipodum,* to the small fish *Gobiomorphus gobioides, Philypnodon* spp. and *Galaxias brevipennis.* The habits of hosts and parasites have been studied and related to the number of parasites found at each stage of the life cycle. The mean trematode population of the eels in any habitat should be the expression of the interrelation between the numbers and habits of the parasites, their vectors and their hosts. Some factors concerned in this large number of variables have been elucidated, though sufficient data are not available for a full analysis. The following records are presented, however, as an indication of how the population balance is maintained in Canterbury waters—Heathcote, Selwyn and Cass Rivers and Lake Sarah. The attempt to correlate environment with population density and behavior of host and parasite seems useful in trematode studies.

The Heathcote River is evenly-flowing with few backwaters and has a bed varying from shingle to silt. It is 1 to 2 feet deep. The Selwyn River, 2 miles from its mouth, alternates shingle rapids with sandy backwaters and pools with shallow margins where silt deposits. Ecologically the Cass River is similar to the Selwyn. One of its branches, the Hut Stream, is, however, narrow, rapid and relatively free of backwaters. Lake Sarah is a mountain lake with a shingle bottom in the shallow parts, while there is a raupo swamp at the west end.

Stegodexamene and *Telogaster* were not found in specimens of *Anguilla dieffenbachii* or *A. australis* till the eels reached a length of 35–40 cm. This is partly the result of the rare infestation of gobiids less than 2 cm. long with metacercariae; and partly of the difficulty a 30 cm. eel would have in swallowing a gobiid of more than 2 cm. length. Equally important is the change of behavior in eels shortly after they have grown a full covering of scales at 30 cm. length. Until that time the young eel spends its life buried in mud under stones or in crevices in the stream bed, and feeds on the larvae of Trichoptera and Ephemerida, and on small crustaceans (cryptozoic behavior). The few specimens of the trematode *Coitocaecum anaspidis* found in young eels come from ingesting the 2–3 mm. long amphipod, *Paracalliope,* which carries *Coitocaecum* metacercariae. This trematode is absent or rare in older eels which feed upon larger animals. Young eels swim openly during the mass upstream migrations that take place during floods, otherwise they are hidden even at night.

Eels over 35 cm. long, however, eat fish of all sizes, birds, larval and adult insects, worms and crustaceans as large as *Paranephrops* (10 cm. long—the freshwater crayfish). Although eels mainly lie in hiding by day, they seek food actively

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in open water at night. It is at this stage that *Gobiomorphus* and the related gobiid *Philypnodon* are ingested. The two species of metacercaria simultaneously acquired from these intermediate hosts are released from their cysts in the eel's duodenum. Probably some form of chemotropism then determines that the *Stegodexamene* remain in the duodenum while the *Telogaster* move down to the jejuno-ileum and rectum. The boundary between the two trematode habitats is remarkably clear cut. Both species show a marked thigmotropism which causes them to cling to the gut wall by means of the oral sucker and when chyle is placed in a dish, they quickly migrate to the surface of the chyle.

At attempt was made (Macfarlane, 1936) to determine the age at which the change of habit in eels took place and the findings were confirmed by Cairns (1941). Frost's results (1945) for *A. anguillae* are substantially the same. In October,

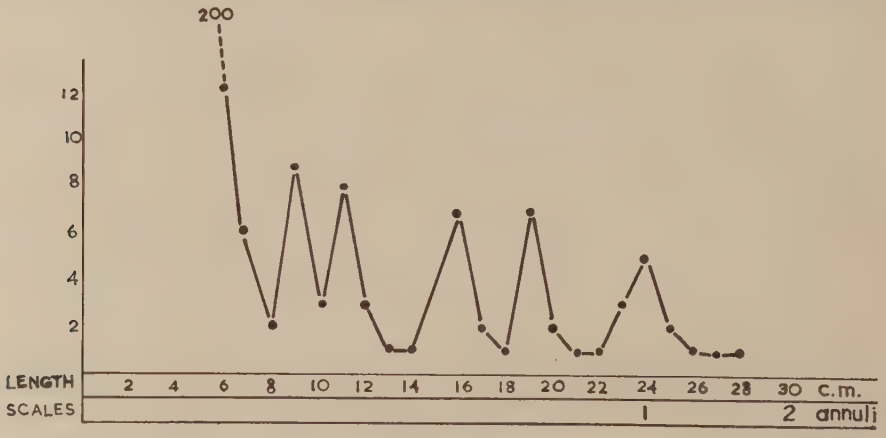

FIG. 1. Frequency of distribution of length of 280 young eels (*Anguilla australis*). The ordinate represents the number of specimens occurring in each length group of the abscissa. It is likely that each peak represents a year's growth, so that scales appear at 7–8 years and adult behavior begins at 9 years from hatching .

elvers of *Anguilla australis,* which have very recently reached fresh water, are 5.5 to 6.8 cm. long with a mean length of 6.15 cm. (200 specimens.) Otolith bands indicate that they are 2 years old. The length-frequency curves of 280 young eels taken in summer (Fig. 1) suggest that if the elver at 6.2 cm. enters fresh water in its third spring after a spring hatch, it is $7\frac{1}{2}$ years old (22-25 cm. long) when its first scales appear in the lateral line caudally. It would then be in the ninth year that the transition to adult life and acquisition of parasites took place, at a length of 35–40 cm. Length frequencies and scale growth indicate that the breeding migration to sea at a length of 70–90 cm. occurs in the twelfth to fourteenth year from hatching. This would mean that there are normally only 3–5 years during which infection occurs.

As the fish grew older there was some reduction in the parasite burden. Environmental differences had a marked effect upon the number of parasites carried by adult eels. Amongst samples of eels from 50 to 90 cm. long, the greatest rate of infection occurred in Lake Sarah (average of 3 eels, 795 parasites per adult eel) with successively fewer parasites in the slow-flowing river (Selwyn, average of 16

eels, 267), the faster flowing stream (Heathcote, average of 21 eels, 156) and fewest in the small rapid stream harboring many eels (Hut Stream, only 0.3 parasites were found per eel). This graded distribution is associated with the availability of the gobiids as food. In each of the lake eels the remains of gobiids were found in the alimentary canal, and they were found in 2 out of 16 Selwyn River fish, but only 1 of 21 Heathcote River eels contained gobiid remnants. In an otherwise suitable stream for the trematode cycles (Hut Stream) where the gobiid vector was rare, the eels were very lightly parasitised. There were 0.3 *Stegodexamene* per eel in this stream in 12 eels, while in *Potamopyrgus* these trematodes occurred in 0.7 per cent of 510 specimens. Gobiids were few so that the heavy population of eels and *Potamopyrgus* was only lightly infected.

The Secondary intermediate hosts

Gobiomorphus gobioides and *Philypnodon* spp. are the small fish most heavily infected with metacercariae of *Stegodexamene* and *Telogaster*. *Galaxias* spp. and *Salmo trutta* fingerlings are lightly infected.

The habits and growth of the gobiids determine the extent of their trematode infection. Young fish (1 to 2 cm. long) may be found at all seasons, but there is a distinct spring maximum in breeding. Till the gobiid is 4.0 cm. long and in its second year, it rests openly by day in the shallows supported on its pectoral fins and tail (phanerozoic behavior). Its food includes *Paracalliope fluviatilis* from which it acquires the trematode *Coitocaecum anaspidis* (Macfarlane, 1939).

Specimens of *Gobiomorphus* over 4.0 cm. long become cryptozoic and lie hidden like young eels in the mud or in the crevices of the river or lake shingle beds. Two rivers which alternated rapids and overhung banks (for cryptozoic fish) with wide sandy shallows (where phanerozoic fish are found) were examined. The mean parasite population of each fish was estimated and the ratio of *Stegodexamene* (S) to *Telogaster* (T) determined $\left(\frac{T}{S}\right)$. In the Selwyn River the phanerozoic gobiids (12 fish up to 4.0 cm.) averaged 8 *Telogaster* metacercariae to 5 *Stegodexamene* on each fish $\left(\frac{T}{S} = 1.6\right)$. Cryptozoic specimens (13 fish 4 to 8 cm. long) carried a mean of 29 *Telogaster* cysts each, compared with 44 *Stegodexamene* $\left(\frac{T}{S} = 0.67\right)$. This reversal in proportion with increasing infestation was observed in another similar stream (the Cass), and it also held for the cryptozoic and phanerozoic stages of *Galaxias brevipennis* in the same environments. This means that larger numbers of *Stegodexamene* succeeded in establishing themselves in the cryptozoic semisubterranean fish than in those abroad in the shallows of the stream. It may be explained by the mode of progression of the cercariae. The cercaria of *Stegodexamene* has an oral sucker and an acetabulum with which it loops thigmotactically along the substratum. When it swims it does so continuously, in a series of waves that cause it to move horizontally. It is not such an active swimmer as *Telogaster* nor has it a marked positive phototropism. As a result it is adapted to parasitise the older animals living in the shadows and interstices of a shingle bed, where the gobiids are attacked from below or from the side. Mean parasitisation increased with age.

The cercaria of *Telogaster* on the other hand is a vigorous swimmer which lacks an acetabulum so that it has no power of terrestrial locomotion. It is strongly attracted to light, and in swimming, it moves actively up to the surface of the water and falls passively downwards. The phanerozoic gobiids tend thus to be more readily parasitised as they rest in the open; and the predominance of *Telogaster* metacercariae bedded in the dorsal surface indicates a frequent attack from above through the dorsal fin. No reversal in ratio from 10.3 with increase of size was found in Heathcote fish, where all the gobiids are virtually phanerozoic, for no specimens over 5 cm. long were obtained. With increasing age, increasing numbers of parasites live in the gobiids in all environments. These differing habits of hosts and parasites are represented diagrammatically in Fig. 2 for the Selwyn River, which is slow-flowing.

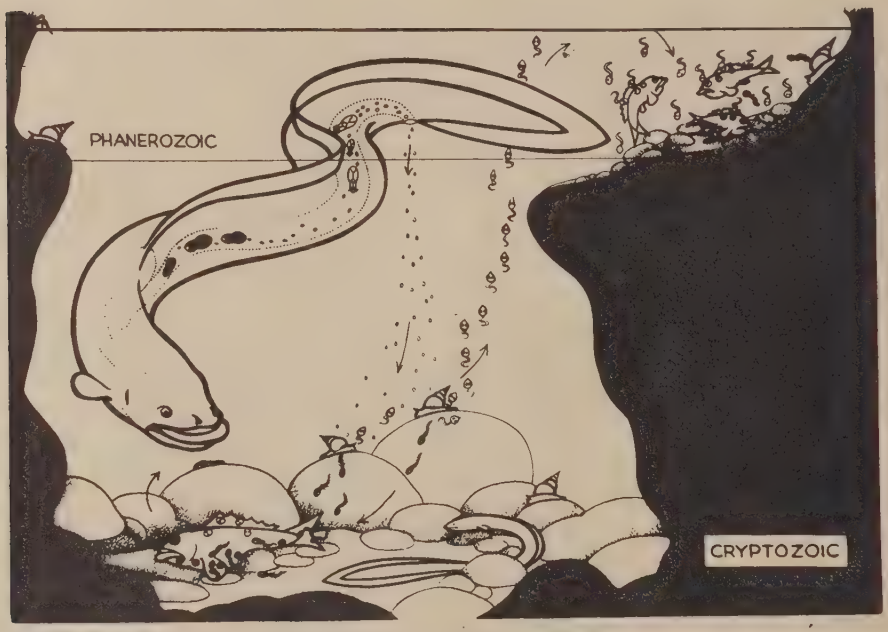

FIG. 2. Diagram summarizing the host–parasite interrelations.

In the deeper water are the cryptozoic young eel and the older gobiids over 4 cm. long. Young gobiids inhabit the shallows phanerozoically. The adult eel is phanerozoic, but it eats both old and young gobiids. *Stegodexamene* (black) infects the foregut and *Telogaster* the hind gut. *Potamopyrgus* is present at all levels and from it emerge *Telogaster* cercariae (white) which swim upwards to drop down on the young gobiids. *Stegodexamene* cercariae (black) crawl over the substrate or swim horizontally to infect more particularly the cryptozoic gobiids.

The numerical comparison in Table 1 of parasite population of all hosts in the cycle in the Heathcote River near its source, and in Lake Sarah near Cass, is a summary of the host–parasite relations. *Gobiomorphus* is the central figure, receiving parasites from molluscs and handing them on to eels. Average infestations per specimen are given for the fish, and rates of infestation per mille for the molluscs.

From these data it appears that (i) the river eels carried fewer trematodes than the lake eels. (ii) *Telogaster* was relatively more plentiful in the river than in the

TABLE 1

	Number	Size (length)	Average parasitism		Ratio T/S
			Telogaster	*Stegodexamene*	
HEATHCOTE RIVER					
Potamopyrgus	1118	4.5–6.0 mm.	22.5%	1.8%	12.4
Gobiomorphus*	25	2.5–4.0 cm.	15.4	1.5	10.3
Anguilla*	21	40–120 cm.	132	24	5.5
LAKE SARAH					
Potamopyrgus	857	4.5–6.0 mm.	9.5%	27.2%	0.35
Gobiomorphus*	16	2.0–4.0 cm.	1.7	2.6	0.66
Anguilla*	3	40–120 cm.	328	467	0.70

* Average parasitisation per fish.

lake. (The Selwyn River with lakelike pools in its course showed intermediate ratios of 1.6 for *Gobiomorphus* and 2.8 for *Anguilla*.) (iii) The degrees of infestation of host and vectors ran parallel in each environment.

The large cryptozoic gobiids were rare in these two localities. The *Gobiomorphus* specimens from the Heathcote River were more heavily parasitised than those from the lake, whereas the eels from the lake carried more parasites than those of the river. This most likely follows from the greater part that gobiids take in the food supply of lake eels. Small gobiids are extremely numerous on the littoral. Each of the lake eels had a gobiid in the stomach and scales in the intestine, so that the small fish are a constant source of food. River eels contained mainly insects and rarely any trace of fish. The trematodes parasitising lake eels were smaller than usual (0.5–0.8 mm. long, normal 1.5–3.5 mm.), which suggests that they were acquired from young phanerozoic fish in which they had not developed far as metacercariae. The similar ratios of *Telogaster* to *Stegodexamene* in the lake bullies and eels (0.67 and 0.7) coincides with the observation that the eels ate many young gobiids in the lake, where the fish are numerous and readily caught. In the still water of the lake, *Telogaster* and *Stegodexamene* cercariae apparently parasitised the gobiids with equal ease irrespective of the swimming habits of cercariae.

In the Heathcote River the predominantly insect-eating eels were lightly infected and carried relatively more *Stegodexamene* than the gobiids. Since the cercariae passed in nearly the same ratio from molluscs (12.4) to phanerozoic gobiids (10.3), the eels' infection (5.5) was probably due to their eating older cryptozoic fish from another locality. These would carry more *Stegodexamene* metacercariae. In moving water the difference in cercarial locomotion showed up in different rates of young and old bully infections. With increasing age the gobiids became more heavily infected (Table 2).

TABLE 2

	Eels		Gobiids 0–4 cm.			4.1–8 cm.
	T/S	Mean No. parasites	T/S	Mean No. parasites	Abundance	Mean No. parasites
Lake	0.7	795	0.67	4.2	++++	7.5
Slow River	2.8	267	1.60	12.7	++	73.1
Faster River	5.5	156	10.0	17.2	+	36.8

The correspondence of populations of parasites in hosts, varying with environment, is summarized in Table 2.

There are many factors and variables in the host-vector bionomics that have not been accounted for: but the ratios given indicate that a quantitative approach to the question is possible.

The Molluscan Vector.

Potamopyrgus antipodum, P. badia and *P. corolla* inhabit both lakes and rivers and appear to be equally susceptible to invasion by *Stegodexamene* and *Telogaster. P. antipodum* is, however, largely a river inhabitant (84 per cent of all *Potamopyrgus* spp. in the Heathcote River) whereas *P. badia* is a lacustrine species (69 per cent of *Potamopyrgus in* Lake Sarah. On river silt 400-1,000 *Potamopyrgus* to 5 cm. × 2 sq. decimeters were obtained. The population density is less on aquatic vegetation and on river shingle. In all the localities studied, however, there are more gastropod hosts than necessary for the completion of the life cycle of the trematodes, and the molluscan hosts could not be considered a limiting factor to the trematode population.

Breeding of *Potamopyrgus* spp. continues throughout the year with a maximum in summer, though there is a small winter increase in reproduction rate.

The degree of total infection increases with the size of the molluscs, as may be seen from the following counts:—

Heathcote River (July and August)

Percentage of *Potamopyrgus* parasitised

Length	Number	*Telogaster* and *Stegodexamene*	All species of cercaria
2.5–3.5 mm.	181	0.0%	0.5%
3.6–4.5 mm.	299	1.0%	8.3%
4.6–6.0 mm.	234	2.7%	15.8%

In the Heathcote River the proportion of *Potamopyrgus* over 4.6 mm. long varied with season from 4.3 to 17.2 per cent.

SUMMARY AND CONCLUSIONS

The populations of trematode parasites in eels living in a lake, two slow flowing rivers and a rapid stream have been compared and correlated with the age, habits and number of the hosts.

The molluscan host is abundant and is an invariable in the ecological pattern. The degree of infection of eels is related, then, to

(a) The age and habits of the eels, which change from cryptozoic to phanerozoic life at 35 cm. long (9 years of age) and at that age become parasitised. They cease to eat small food animals and begin to eat the intermediate host gobiids.

(b) The habitats of the eels. Eels become heavily infected in the lake and lightly infected in fast rivers. This is related to the numbers of the gobiid.

(c) The behavior of the gobiid vector, which changes from phanerozoic to crytozoic habit at 4.0 cm. long (2 years of age). In quiet waters the immature phanerozoic gobiids are eaten by the eels, while in faster streams the larger vectors are eaten.

(d) The behavior of the cercariae. *Stegodexamene* cercariae are thigmotropic and infect the large cryptozoic gobiids, while *Telogaster* cercariae are positively phototropic and infect more of the free living young intermediate hosts.

Quantitative evidence is given for the interrelation of the parasite-host-vector behavior patterns with the environment.

ACKNOWLEDGMENT

Professor E. Percival of Canterbury University College provided help and advice for which I am most grateful. Miss P. F. M. Sinclair kindly drew the diagram of the host-vector behavior patterns.

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THE EFFECTS OF VARIOUS BACTERIA AND THEIR METABOLITES ON GROWTH OF *TRICHOMONAS VAGINALIS IN VITRO*[1]

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INTRODUCTION AND HISTORICAL REVIEW

Trichomonas vaginalis has received perhaps less attention than any of the other parasitic protozoa that have thus far been isolated in pure culture and studied for their physiological requirements and the effects of other microorganisms on their growth. This investigation was prompted by the lack of such information in respect to *T. vaginalis,* and also by some indication, in work with other trichomonads, that certain bacteria might play an antagonistic, i.e., antibiotic, role.

To date, studies of this type have been concerned more with *Trichomonas foetus* than with species parasitizing man. Early attempts to cultivate *T. vaginalis* free of bacteria were unsuccessful (Lynch, 1915, 1922; Davis, 1929; Andrews, 1929; Bland, Goldstein, Wenrich, and Weiner, 1932). Apparently the first successful isolation of this organism was by Trussell (1940). Later Johnson, Trussell, and Jahn (1945) found that exposure of *T. vaginalis* in vaginal discharge to 5,000–10,000 units of penicillin enabled them to isolate the flagellate from accompanying bacteria.

After cultivation of trichomonads without bacteria was accomplished, attention was directed toward devising media that not only would support good growth but also contain known components in predetermined concentrations and thus would serve as tools for physiological studies. Among such media are those of Schneider (1942) and its modification used by Johansson, Morgan, and Winkler (1947) in the cultivation of *T. foetus.* Johnson and Trussell (1943) devised for the growth of *T. vaginalis* a medium known as the C. P. L. M. medium based on several investigations on the physiology of that species (Trussell and Johnson, 1941; Johnson, 1942; Kupferberg and Johnson, 1941; and Trussell and Plass, 1940). Sprince and Kupferberg (1947) modified the original C. P. L. M. medium replacing many of the ingredients with better defined substances. This modification, designated as trypticase medium by Sprince and Kupferberg, has been utilized for experimental work in the present study. Shaffer, Ryden, and Frye (1949) devised a clear "preconditioned" thioglycollate medium in which the trichomonad as well as certain other parasitic protozoa grew abundantly. This medium has been used in the present study for maintenance of stock cultures of *T. vaginalis.*

Although trichomonads are associated with various bacteria in the host and were not cultivated apart from them until fairly recently, little work has been done toward determining the effects of bacteria on flagellate growth *in vitro.* Most of the available information of this type concerns *T. foetus* (Morgan, 1942; Plastridge, 1943;

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Williams and Plastridge, 1946; Johansson, Morgan, and Winkler, 1947; and Ford and Morgan, 1948). A search of the literature fails to reveal such studies dealing with *T. vaginalis*.

MATERIALS AND METHODS

Since any organism long maintained by *in vitro* cultivation may become altered physiologically, three strains of *T. vaginalis* that had been isolated for varying periods of time were investigated comparatively. These were: the Vanderbilt strain, obtained through the courtesy of Eli Lilly Co.; one isolated more recently at Indiana University and obtained also from Eli Lilly Co., and one isolated at the beginning of this study from an active case of vaginitis in Lafayette, Indiana. However, comparative studies in the early experiments showed no observable differences in the three strains and since the Vanderbilt strain has been widely investigated it was the principal one employed here. When obtained, the first two strains were growing on Shaffer-Frye medium while the locally isolated one was rendered free of multiplying bacteria by the use of antibiotics during the initial transfers in that medium.

Stock-culture Agar (Difco) was used to maintain all species of bacteria investigated except *Streptococcus lactis* which was grown in litmus milk. When used in connection with *T. vaginalis*, bacteria were transferred from these stocks to liquid thioglycollate medium or to trypticase medium of Sprince and Kupferberg (1947). Periodic examinations were made to determine that the bacteria remained in pure culture.

Stocks of *T. vaginalis* were maintained in the liquid medium of Shaffer, Ryden, and Frye (1949). Since this medium must be overlaid with vaseline to maintain anaerobic conditions and essential ingredients include dead or attenuated bacterial cells and/or their metabolites, it was not desirable for use in studying effects of specific bacteria or their products on the growth of *T. vaginalis*. After several other media were tested for their ability to support luxuriant growth of the flagellate in the absence of multiplying bacteria, that devised by Sprince and Kupferberg (1947) was found to be best for the purpose at hand. It had the advantages of: (1) having an almost entirely synthetic composition, hence allowing a more accurate study of nutritional needs, (2) being liquid, thus permitting quantitative transfers, (3) having the anaerobic state maintained by a cysteine hydrochloride indicator system, thus eliminating the need for the inconvenient overlay of vaseline, and (4) containing no bacterial cells or their metabolites. This medium was modified only to the extent of substituting horse serum for human serum. After preparation, tubes were incubated for sterility before inoculation with *T. vaginalis* or bacteria.

Hydrogen ion concentrations were determined with a Beckman Model H-2 pH Meter and oxidation-reduction potentials were measured with the same instrument adapted for that purpose. When potentials were to be determined, cultures were prepared in such a manner that duplicate electrodes were used in each tube and left in place throughout the experiment so that readings could be made at any time without disturbing the culture. All e. m. f. readings were recorded as millivolts determined with the platinum electrode against a HgCl half-cell.

To obtain filtrates of bacterial cultures, various species were inoculated into several tubes of trypticase medium which were incubated at 37° C. for varying periods, and then filtered through a sterile, ultra-fine, glass filter. Filtrates thus obtained were tested for their effect on growth of *T. vaginalis*.

Flagellates were counted with a haemacytometer. In experimental cultures, the viscosity of the medium interfered with obtaining homogenous suspensions. Fairly uniform mixtures resulted, however, when a sterile pipette was introduced into the culture and the medium carefully withdrawn and then permitted to run back into the tube four or five times. This procedure apparently did not affect the reproductive or other metabolic activities of the flagellate and gave consistent counts.

An inoculum of approximately 50,000 active flagellates from a 48- or 72-hour culture was standard throughout. After transfer of this number to trypticase medium and during incubation at 37° C., counts were made at regular intervals, usually each 24 hours but at 6- and 12-hour intervals in some experiments. In all cases, only motile flagellates were counted and duplicate counts were made. Aseptic techniques were maintained at all times and cultures that became contaminated were discarded.

In all experimental cultures involving *T. vaginalis,* a minimum of 10 replicates was observed and counted. Variation in count among these replicates was not significant. Because of the uniformity in the number of flagellates occurring among the replicates in each test, only averages are reported and discussed in this paper.

OBSERVATIONS AND DISCUSSION

Normal or Control Population Curve of *T. vaginalis*

The normal or control population growth curve (Fig. 1) depicts the flagellate

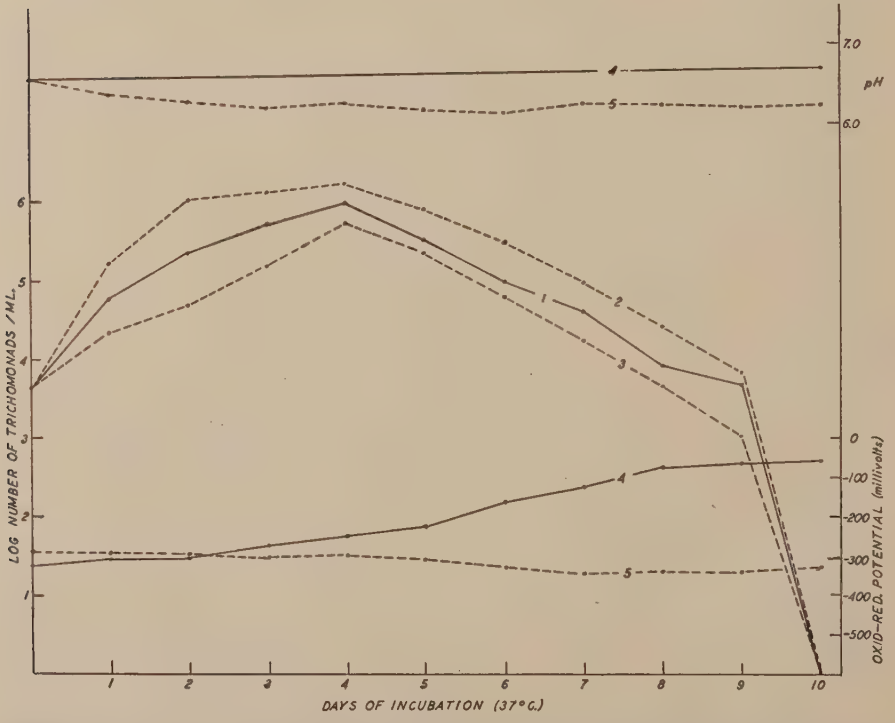

FIG. 1. Population, oxidation-reduction potential, and pH curves for normal or control cultures of *Trichomonas vaginalis.* 1. average, 2. maximum, and 3. minimum populations; 4. uninoculated, and 5. inoculated medium.

population developing when 50,000 organisms were inoculated into 10 ml. of the medium of Sprince and Kupferberg containing no bacteria or their metabolites. Control pH and oxidation-reduction potential curves represent determinations taken from such cultures. These curves are based on readings made at 24-hour intervals from the time of inoculation until the organisms disappeared or became inactive and provide a basis for observing and evaluating the effects of experimental procedures. Fig. 1 is based on over 100 individual cultures, including a preliminary series and controls run with various experiments. However, points beyond the 120th hour on the population curve are based on smaller numbers of determinations since the controls for experimental cultures were not counted beyond the time that living flagellates persisted in the experimental tubes.

The control population curve showed no initial stationary or lag phase; the fresh medium favored immediate growth and multiplication of the flagellates when these were inoculated from stock cultures at the peak of such activity. Thus the number of organisms increased logarithmically from the time of inoculation until 60 to 72 hours of incubation. This phase was followed until about the 84th hour by a period of negative growth acceleration. Then, until shortly after the 96th hour, there occurred the maximum stationary phase during which the population attained a peak averaging almost 1,000,000 flagellates/ml. Following this peak the number of flagellates began to decline and this phase continued until living ones disappeared by the 240th hour. This period may be referred to as the phase of decline and death.

Effect of Various Bacteria on Growth of *T. vaginalis*

It was found that various species of bacteria affect *T. vaginalis* in three general

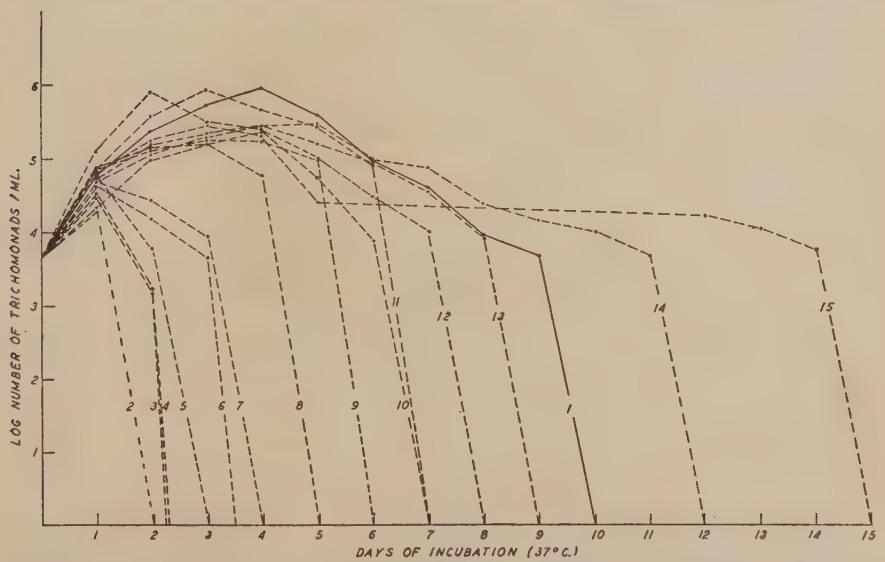

FIG. 2. Growth of *Trichomonas vaginalis* in the presence of various bacteria. 1. control, 2. *Escherichia coli*, 3. *Aerobacter aerogenes*, 4. *Pseudomonas aeruginosa*, 5. *Salmonella schottmülleri*, 6. *Proteus mirabilis*, 7. *Salmonella paratyphi*, 8. *Brucella suis*, 9. *Streptococcus lactis*, 10. *Pseudomonas fluorescens*, 11. *Alcaligenes faecolis*, 12. *Sarcina lutea*, 13. *Bacillus subtilis*, 14. *Staphylococcus albus*, and 15. *Staphylococcus aureus*.

ways although these intergrade (Fig. 2). With certain bacteria, flagellate multiplication is inhibited and *T. vaginalis* persists in the culture only a short period of time (*Escherichia coli,* 48 hours; *Aerobacter aerogenes,* 52 hours; *Pseudomonas aeruginosa,* 52 hours; *Salmonella schottmülleri,* 72 hours; *Proteus mirabilis,* 84 hours; *Salmonella paratyphi,* 96 hours). With another group of bacteria, either flagellate reproduction is inhibited to a less degree or, rarely, its multiplication is enhanced but always with a decrease in the life span of the culture (*Brucella suis,* 120 hours; *Streptococcus lactis,* 144 hours; *Pseudomonas fluorescens,* 168 hours; *Alcaligenes faecalis,* 168 hours; *Sarcina lutea,* 192 hours; *Bacillus subtilis,* 216 hours). With a third group of bacteria, *T. vaginalis* is enabled to persist longer than in the bacteria-free controls (*Staphylococcus albus,* 312 hours; *Staphylococcus aureaus,* 360 hours).

Observations on *T. vaginalis* when grown with certain bacteria are in agreement with those of Johansson, Morgan, and Winkler (1947) in a similar study of *T. foetus*. Thus the effects of *E. coli* and *A. aerogenes* on the two flagellates are much the same. In the present study it was found that other enteric bacteria such as *S. schottmülleri* and *S. paratyphi* also inhibited the flagellate and caused its death within 72 and 96 hours respectively. On the other hand, the writer found that *Ps. aeruginosa* inhibited growth of *T. vaginalis* and caused its death within 52 hours, whereas Johansson, Morgan, and Winkler reported that this bacterium stimulated the growth of *T. foetus* and permitted the flagellates to remain active almost as long as in control cultures. Present results with *Ps. aeruginosa* are more in accord with those of Williams and Plastridge (1946) who found that this species destroyed *T. foetus* within 48 hours. Results with *B. subtilis* and *S. lutea* agree essentially with the observations of Johansson, Morgan, and Winkler in comparable experiments using *T. foetus*. When *T. vaginalis* was grown with *S. albus* and *S. aureus,* it was observed that the life of the flagellate was prolonged, whereas Johansson, Morgan, and Winkler found that these staphylococci destroyed *T. foetus* within about 88 hours. Williams and Plastridge reported that *S. aureus* destroyed *T. foetus* within 24 hours.

It is not surprising that under the conditions of this and the previous studies response of *T. foetus* to bacteria may differ from that of *T. vaginalis*. It should be emphasized that not only may it be expected that the two, being distinct species from different hosts, may differ in their metabolic needs but also that the media used were quite dissimilar in the various studies.

After it was observed that species of bacteria differed in their effects on the growth of *T. vaginalis,* the reasons for these effects were sought by investigating such factors as changes in pH, shifts in oxidation-reduction potential, the production of antagonistic substances, and competition for nutrients or alteration of nutritional factors.

Studies on Hydrogen-ion Changes

The basal medium was adjusted to pH 6.0 and, upon addition of ascorbic acid-sodium bicarbonate solution and horse serum, the final pH was 6.5–6.6. Readings of pH were made at regular intervals on both control and experimental cultures as well as uninoculated medium. As shown in Fig. 1, the pH of cultures of *T. vaginalis* alone gradually decreased during the first 144 hours, the rate of decrease being

0.05 to 0.1 pH unit each 24 hours. From the minimum at 144 hours, there was a shift in the opposite direction until an equilibrium at about pH 6.25 was established. In all cases, the pH of uninoculated medium shifted gradually toward neutrality from an original of 6.5–6.6 and established an equilibrium at about pH 6.8.

In experimental cultures it was observed that the bacteria *S. albus, S. lutea, B. suis, S. schottmülleri, E. coli,* and *A. aerogenes* when grown alone or with *T. vaginalis* changed the pH of the trypticase medium very little, in no case exceeding 0.25 pH unit. *S. albus, A. aerogenes,* and *E. coli* caused a slight shift toward the acid side while the pH increased slightly with *S. schottmülleri* and *S. lutea. B. suis* caused a slight increase in pH of the medium followed by a return to approximately the original value.

A more pronounced decrease in pH was noted in cultures of *S. lactis, S. paratyphi, P. mirabilis, S. aureus,* and *Ps. aeruginosa.* In each of these, there was a drop of from 0.5 to 0.6 pH unit within the first day or two. This effect was most pronounced in cultures of *S. paratyphi* in which the pH decreased to 5.95 during the first 24 hours and remained near that value through the 96th hour. In the others, there was a gradual return to near the original pH of the medium.

In contrast to the above bacteria, *Ps. fluorescens, A. faecalis,* and *B. subtilis* brought about rather pronounced shifts toward the alkaline side. This effect was greatest in the case of *Ps. fluorescens* which caused an immediate and rapid shift to a pH of 7.3 within the first 48 hours. After that time, the change became more gradual and the pH reached an equilibrium of about 8.0 at the 96th hour. According to Johnson (1942), this is outside the range tolerated by the flagellate. However, with *Ps. fluorescens* the flagellate death occurred after the 96th hour when the pH of the medium was beyond that range. This bacterium was the only one with which there was any correlation between pH change and growth of the trichomonad. Although *A. faecalis* caused a shift to an alkaline reaction beyond the point tolerated by the flagellate, the change was more gradual than with *Ps. fluorescens,* and reached a maximum of only pH 7.65 at 168 hours, which was after the accelerated death phase had begun. *B. subtilis* caused a more moderate increase in pH of the medium, a maximum of 7.0 being attained at the end of 192 hours. In experimental cultures, pH readings were not continued beyond the point at which living trichomonads disappeared from the medium.

In summary, it may be said that although certain bacteria altered the pH of the medium to a point unfavorable to *T. vaginalis,* there was no general correlation between this change and the effect of various bacteria on the growth of the flagellate.

Studies on Oxidation-Reduction Potentials

It has been shown that *T. vaginalis* is a facultative anaerobe (Johnson, 1942). Chang (1946) reported that there was a correlation between the growth of *Endamoeba histolytica* and the oxidation-reduction potential of the culture medium. He found that abundant growth of the amoebae occurred in a medium with a potential of −275 to −425 millivolts, and that there was a direct correlation between the growth of certain bacteria and oxidation-reduction potential changes brought about in the medium by these organisms. Although the significance of these potentials to parasitic protozoa is debatable, it was thought desirable to determine if there was

any correlation between them and growth of *T. vaginalis* in the presence of various species of bacteria.

The uninoculated trypticase medium was found to have a potential of from – 210 to – 330 mv. which gradually shifted toward zero, as far as – 50 mv. upon standing 10 days. A change in the opposite direction was observed in cultures supporting the growth of *T. vaginalis*. In bacteria-free ones (Fig. 1) this was gradual with very little change during the first 120 hours, shifting from – 285 to – 307 mv. There was a drop to – 342 mv. by the 168th hour and after the 192nd hour the potential leveled off at – 330 mv.

In all cultures in which *T. vaginalis* was grown with bacteria, oxidation-reduction potential changes were much the same, and since these changes were not significantly different from those of the control (Fig. 1), they are not shown. The only differing results were obtained with *B. subtilis,* with which there was little change in potential throughout the 10-day period.

As with observation on pH, no correlation could be established between oxidation-reduction potential changes and effects of various bacteria on growth of *T. vaginalis*. The writer's observations are in agreement with those made on *E. histolytica* by Jacobs (1950) who was unable to find a definite correlation between low oxidation-reduction potentials maintained by various bacteria and their positive effect on growth of the amoeba.

Studies of Bacterial Filtrates

T. vaginalis was next grown in medium to which were added filtrates of various bacterial cultures, to determine whether the effects previously observed might be due in part at least to bacterial metabolites.

In these experiments, filtrates of 24-, 48-, and 72-hour cultures were prepared and added to the basal medium in amounts of 1.0 and 2.5 ml. When more filtrate was used, the results were inconclusive because agar, as well as bacteria, was removed by the filtration process, and its final concentration was too low. Sprince and Kupferberg (1947) had shown that agar is apparently an essential ingredient of the medium if good trichomonad growth is to be obtained, although it is not known whether this is because the effect is a nutritional one or merely imparts a favorable consistency. Filtrates of *E. coli, A. aerogenes, Ps. aeruginosa,* and *S. albus* were tested for their effects on the flagellate. Whereas *T. vaginalis* disappeared from cultures within 48 hours when *E. coli* and *A. aerogenes* were present, the addition of 1.0 ml. of 48-hour filtrates of these bacteria to the medium resulted in little or no effect upon the growth of the flagellate as compared with bacteria-free controls. Essentially the same results were obtained when filtrates of 24- or 72-hour cultures and 2.5 ml. amounts were used. Also, *Ps. aeruginosa* and *S. albus* gave results very similar to those observed with *E. coli* and *A. aerogenes* filtrates.

These results indicated that in no case were the effects of the bacteria due to their metabolites. The population of flagellates at any given time was well within the range of variability observed in the controls (Fig. 1) and the average was very slightly below that of controls. These findings are not in agreement with those of Johansson, Morgan, and Winkler (1947) who reported that bacteria or their filtrates had similar effects on growth of *T. foetus*. However, they gave no details of technique and this lack of agreement may be due to differences in procedure.

To exclude the possibility that the filtering process had altered bacterial metabolites, a qualitative study of the trichomonad in the presence of living bacteria was made. Flagellates were carefully placed at the bottom of the tube of medium and the bacterial inoculum was layered at its surface. The culture was not mixed and no counts were made. By observation made through the side of the tube with a microscope it could be determined whether or not the flagellates were living and active. Because of the viscosity of the medium, the bacterial cells and the trichomonads did not become generally intermixed for several days. Under these conditions, the trichomonad continued active growth for a considerable time beyond that in mixed bacterial cultures and there was no indication that bacterial metabolites that might be expected to diffuse through the medium, had any effect on the protozoan.

Nutritional Studies

After eliminating the likelihood that bacteria exerted their effects through their metabolites or by changes in the hydrogen ion concentration or oxidation-reduction potential of the medium, there remained the possibility that nutritional changes might be responsible for the results obtained. This was tested by a series of experiments in which cultures were reinforced with components of the trypticase medium, either singly or in combinations. These were added either at the time of inoculation with bacteria and flagellates, or later to replace components that might have been depleted. With the three species of bacteria that showed the strongest inhibiting effect on *T. vaginalis,* their inocula were diluted approximately 1 : 100,000 to delay massive overgrowth by the species and hence their utilization of components of the medium. This probably explains the higher flagellate count for controls in this series than in cultures initially inoculated with a much larger number of bacteria (Fig. 2).

The following ingredients were made up singly or in combinations in concentrations such that the addition of 0.1 ml. of any one would double its concentration in the tube of trypticase medium: 1. serum (added in amounts of 1.0 ml. instead of 0.1 ml.), 2. vitamin B complex, 3. amino acids, 4. purines and pyrimidines, 5. choline Cl and inositol, 6. biotin and folic acid, and 7. maltose. Bacterial and flagellate inocula were then introduced into the medium, enriched with one or more of these substances, the tubes incubated, and counts of the flagellates made at intervals.

Of the above substances, only maltose had any effect on growth of the flagellate in the presence of bacteria as compared with that observed in control cultures which had not been reinforced. Fig. 3 shows the results with *E. coli;* those obtained with *A. aerogenes* and *Ps. aeruginosa* were much the same. When maltose was added in 0.1% (thereby doubling the amount in the original medium), 0.2%, or 0.4% amounts (curves 3, 4, 5, and 7), the peak population density was increased above that of nonenriched cultures with *E. coli* (curve 2). With 0.2% maltose added to the medium the life of the trichomonad was prolonged until the 72nd hour, 24 hours longer than in the nonenriched culture, while with 0.4% maltose, the life of the culture was prolonged until the 9th day.

Further observations were made on growth of *T. vaginalis* in a maltose-reinforced medium but varying the time of inoculation with *E. coli.* The trichomonad was first grown in an unenriched bacteria-free medium for 24 hours, a population

count taken, and the tubes inoculated with *E. coli.* As shown in Fig. 3 (curve 6), the flagellate number was close to that of controls at the end of 24 hours. After *E. coli* was added, the trichomonads continued to increase in number, reaching a peak at the 48th hour. By the 72nd hour, the count decreased rapidly and no living trichomonads were present by the 4th day. The growth of the flagellate began in normal fashion, but after the addition of the bacterium the population curve was altered to resemble more those obtained in other experiments with *E. coli* (Fig. 2).

In a similar experiment with the delayed inoculation of *E. coli* into a 24-hour flagellate culture but enriched with 0.4% maltose (curve 7), it was found that the flagellate population increased as in controls during the 1st 24 hours. After inoculation with *E. coli, T. vaginalis* reached a peak population density almost two and one half times that of the control. Thus, *E. coli* added after 24 hours was not able to

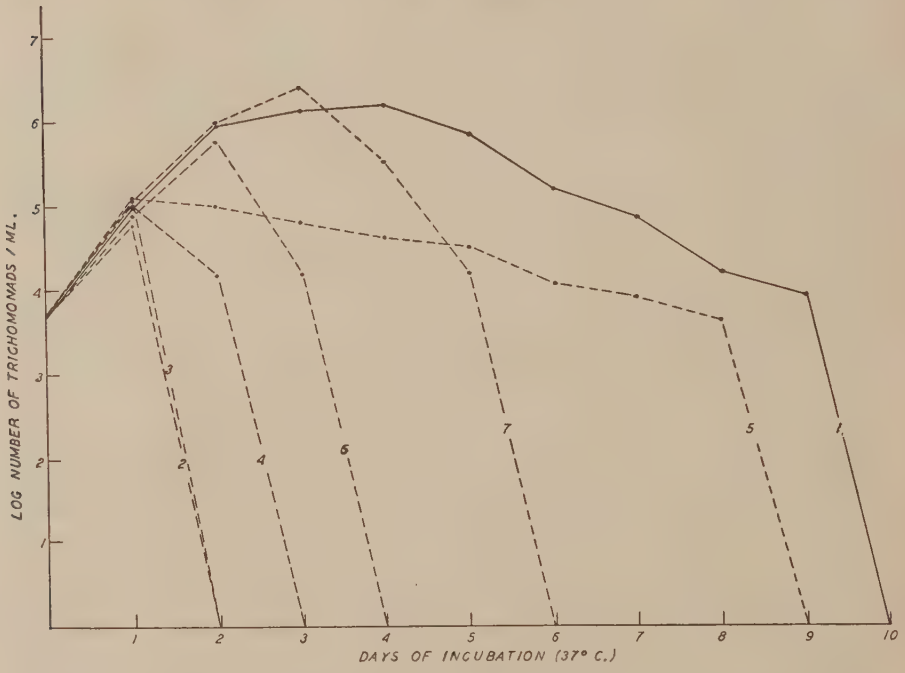

FIG. 3. Effects of varying concentrations of carbohydrate (maltose) and of time of inoculation with *Escherichia coli* on growth of *Trichomonas vaginalis.* 1. control population curve in absence of bacteria and additional carbohydrates; 2. to 5. inoculated with both bacteria and flagellates at same time; 6. and 7. inoculated with bacteria after growth of the flagellates for 24 hours; 2. and 6. basal medium without additional maltose, 3. with 0.1%, 4. with 0.2%, and 5. and 7. with 0.4% maltose added.

affect the population rate of *T. vaginalis* in medium reinforced with 0.4% maltose until the fourth day when the number of flagellates began to decrease rapidly with living ones absent by the end of the 6th day.

Glucose substituted for maltose in the above experiments gave similar results. This observation is in accord with the studies of Trussell and Johnson (1941) who found that only glucose and its polymers were utilized by *T. vaginalis.*

From these experiments, it seems evident that the effects of bacteria on growth of *T. vaginalis in vitro* are nutritional, and that of the various components of the medium, carbohydrate alone is the substance which certain bacteria remove and thus bring about a decline in population of the flagellate. When carbohydrate is added to the medium, or if inoculation with bacteria is delayed, this decline likewise is delayed. The fact that additional carbohydrate in bacteria-free cultures had no significant effect on flagellate population density during the first 48 hours indicates that during that interval, the protozoan is reproducing at a maximum rate for which the carbohydrate content of the basal medium is adequate. However, when abundant carbohydrate is available, the presence of bacteria may actually stimulate growth of *T. vaginalis* above that occurring in the absence of other organisms. This may be due to ingestion of bacteria and their utilization as food by the flagellate.

SUMMARY

The effects of various bacteria on growth of *Trichomonas vaginalis* have been investigated. Three strains of the trichomonad (Vanderbilt, Indiana University, and one isolated locally) were employed, stock cultures being maintained in the medium of Shaffer, Ryden, and Frye, and that of Sprince and Kupferberg being utilized for experimental work.

In their effects, the bacteria fell roughtly into three groups: (1) those which greatly curtailed multiplication of the flagellate and the life of the culture (*Escherichia coli, Aerobacter aerogenes, Pseudomonas aeruginosa, Salmonella schottmülleri, Proteus mirabilis,* and *Salmonella paratyphi*); (2) those having a moderately inhibiting effect or, rarely, enhancing multiplication of the flagellate but always causing some decrease in the life span of the culture (*Brucella suis, Streptococcus lactis, Pseudomonas fluorescens, Alcaligenes faecalis, Sarcina lutea,* and *Bacillus subtilis*); and (3) those prolonging the life of the culture beyond that of bacteria-free controls but with the flagellate population never exceeding that of the controls at any given time (*Staphylococcus aureus* and *Staphylococcus albus*).

Changes in pH and oxidation-reduction potentials gave no indication that the effects of bacteria were due to such changes. With *Pseudomonas fluorescens,* the shift was beyond the alkaline limit tolerated by *T. vaginalis,* but this occurred after inhibition of the flagellate had begun.

Filtrates of bacterial cultures gave no indication that specific metabolites or antagonistic substances were responsible for the effects observed.

Nutritional studies showed that of the various components of the medium, maltose seemed to be the important one for which there is competition between the flagellate and bacteria inhibiting it most. However, in the presence of abundant maltose and such bacteria, the *T. vaginalis* may attain a higher population peak than with maltose alone. When used to enrich the medium, glucose was found to be as effective as maltose in prolonging the life of the trichomonad culture in the presence of bacteria which had an inhibiting effect.

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HELMINTHS OF NORTHWESTERN MAMMALS. PART I. TWO NEW SPECIES OF *HYMENOLEPIS*

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Apparently six species of *Hymenolepis* have been described previously from northwestern mammals: one (Rider and Macy, 1947) from the muskrat; another (Macy, 1947) from the northwest coast bat; and four species (Locker and Rausch, 1952) from various shrews. Two additional species are described in the present paper, one from the mole, *Scapanus townsendi* Bachmann, and the other from the muskrat, *Ondatra zibethica occipitalis* (Elliot).

All cestodes collected were fixed in formalin-alcohol-acetic acid solution. If the tapes were alive when removed from the host, they were relaxed in cold tap-water before fixing. Specimens were stained with either Semichon's acetocarmine or Kornhauser's hematin, dehydrated in alcohol, cleared in terpineol, and mounted in clarite. Since specimens stained with Kornhauser's hematin were unusually favorable for study, serial cross-sections were not required for an adequate study of the tapes. All measurements are given in millimeters unless otherwise stated. The drawings were prepared with the aid of a camera lucida.

We wish to thank the Oregon State Game Commission and state trappers Lee and Homer Taylor for their very kind assistance in obtaining the muskrats utilized in this study. We would like to thank Mr. Ernest Olson, who furnished the moles from which the cestode named in his honor was collected. We also wish to thank Dr. Ralph W. Macy for his great interest in our work and for the use of the facilities of the Reed College Biology Laboratories.

Hymenolepis oregonensis n.sp. (Plate I, Figs. 1-5)

The strobila is serrate and reaches a length of 18-42 cm. in gravid specimens; a maximum width of about 1.2 is attained in ripe proglottids. The segments are always much broader than long. A weakly developed scolex is present; it measures 0.235–0.367 broad. The suckers are oval and protrude from the body of the scolex; they measure 0.133–0.163 long by 0.102–0.122 broad. A prominent rostellum, 0.255–0.357 long, is present. It bears ten hooks which are 0.042–0.048 (average, 0.046) long. The lateral excretory vessels are situated one above the other with the ventral canal measuring about 0.026–0.051 broad. The excretory canals pass ventral to the genital ducts. The genital pores are unilateral and dextral, and are located near the end of the first third of the lateral margin of the proglottid.

The cirrus pouch, a large club-shaped organ, is 0.204–0.336 long by 0.031–0.041 broad and extends well past the excretory vessels into the proglottid. The cirrus is short and is armed with numerous small spines. Both an internal and an external seminal vesicle are present. The latter is the smaller of the two and is characteristically situated near or over the ovary; it measures 0.071–0.112 long by 0.031 0.071 broad. The testes and cirrus pouch develop precociously; they are fully developed when the ovary and vitellaria are only represented by an undifferentiated mass of germinal tissue and much atrophied when the female glands are completely formed. The oval testes are in line across the middle of the proglottid and they measure 0.061–0.102 long by 0.041–0.077 broad. The ovary is broader than long and has three main lobes. It is situated in approximately the mid-region of the proglottid and may be 0.112–0.133 long by 0.306 0.408 broad. The vagina opens ventral to the cirrus pouch and is slightly enlarged at that point. Near its distal end the vagina is dilated, forming a large seminal reservoir which measures 0.082–0.122 long by 0.031–0.051 broad. The vitellaria are posterior to the middle of the ovary; they measure about 0.051–0.082 long by 0.102–0.224 broad. The gravid uterus has many lobes

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which entirely fill the proglottid. The egg is somewhat elongate, with the ends separated from the middle part by slight constrictions; it measures 0.088–0.106 (average, 0.091) long by 0.026–0.034 (average, 0.030) broad. The hexacanth is enclosed in an additional inner, membranous shell which is elliptical in shape and bears a long recurved filament at each end.

Host: Ondatra zibethica occipitalis (Elliot).

Habitat: Small intestine.

Locality: Mud Lake, Fairview, Oregon and the Columbia River, near Claskanie and Portland, Oregon.

Type specimen: Slides bearing the type specimen have been deposited in the helminthological collection of the U. S. National Museum, number 47,598. Additional slides of paratype specimens are located in the authors' collections.

The precocious development of the male genital organs and the unusual morphology of the egg are features which distinguish *Hymenolepis oregonensis* n. sp. from the majority of other related species. However, there are a number of mammalian forms which somewhat resemble the new species. These forms are found to differ from *H. oregonensis* as follows:

Hymenolepis ondatrae Rider and Macy, 1947 (parasitic in muskrats) is smaller in size (80 × 1.25 mm.); the testicular arrangement is triangular; the hooks are variable in number (8–10) and are larger (0.067–0.073 mm.); and the eggs are much smaller (0.030–0.035 × 0.040–0.045 mm.) and have a different shape.

Hymenolepis evaginata Barker and Andrews, 1915 (parasitic in muskrats) has much smaller hooks (0.007 mm.) which are of a characteristically different shape; the testes are arranged in a triangle; the ovary is bilobed; and the eggs are much smaller (0.020 × 0.016 mm.).

Hymenolepis pearsei Joyeux and Baer, 1930, differs in the size (0.069 mm.) and shape of the hooks; the strobila is relatively shorter and broader (100 × 2 mm.); the cirrus pouch is larger (0.420 × 0.060 mm.); the ovary and vitellaria are poral to the midline of the proglottid; and the eggs are smaller (0.065 mm.).

Hymenolepis lanceolata (Bloch, 1782) Stiles, 1896, has fewer (8) and smaller hooks (0.031–0.035 mm.); the testes are all poral to the midline of the proglottid; the rostellum is proportionately much shorter (0.040 mm.); and the cirrus pouch is larger (0.8–1.2 mm.).

Hymenolepis diminuta (Rudolphi, 1819) Blanchard, 1891, is relatively much broader (200–600 × 3–4 mm.); the rostellum is unarmed; and the eggs are smaller (0.060–0.070 mm.) and are quite differently shaped.

Hymenolepis peipingensis Hsu, 1935, is much longer (750+ mm.); the rostellum is unarmed; the cirrus pouch is smaller (0.208 × 0.050 mm.) and does not extend past the excretory canals; and the cirrus is aspinose.

Hymenolepis scalopi Schultz, 1939, has an unarmed rostellum; the testicular arrangement is triangular; the cirrus pouch is smaller (0.125 × 0.045 mm.) and does not extend past the excretory vessels; the cirrus is aspinose; and the eggs are smaller (0.057–0.065 mm.).

Of thirty-three muskrats examined during the 1950–51 trapping season, nineteen were found to harbor *Hymenolepis oregonensis* n. sp. Seventeen of the twenty-six rats from the Mud Lake locality and two of seven rats from the Columbia River were infected with this worm.

Hymenolepis olsoni n.sp. (Plate II, figs. 6–9)

The strobila is up to 36 cm. long with the greatest width (1.58) in gravid segments. The margins of the strobila are serrate and the proglottids are always much broader than long. The

scolex which is scarcely broader than the neck measures 0.106–0.153 broad. The suckers are oval and measure 0.092–0.104 long by 0.060–0.067 in width. The rostellum is fairly long, 0.183–0.216, and is armed with 10–12 hooks which are 0.012–0.018 long. The smaller dorsal and larger ventral excretory vessels pass ventral to the genital ducts; the ventral canal measures 0.041–0.117 broad. The genital pores are unilateral, dextral, and are situated near the end of the first third of the lateral margin of the proglottids.

The cirrus pouch does not (or just) reach(es) the excretory vessels; it measures 0.163–0.224 long by 0.020–0.031 broad. The cirrus is unarmed. Both an internal and an external seminal vesicle are present, with the latter measuring about 0.092–0.173 long by 0.031–0.041 broad. The external seminal vesicle is variable in size and may or may not be larger than the cirrus pouch. The testes are arranged in a narrow triangle with the most aporal one located slightly to the left of the lateral margin of the ovary and the other two situated posterior to the ovary, from which they are separated by the vitellaria. The ovary is broader than long, with three lobes, and is situated slightly poral to the mid-line; it measures 0.082–0.122 long by 0.214–0.306 broad. The vitellaria, which lie just posterior to the middle of the ovary, measure 0.061–0.092 broad by 0.051–0.082 long. The gravid uterus has many lobes and occupies the entire proglottid. The egg is oval, with apparently two additional inner membranes enclosing the hexacanth. The brittle, outer shell is 0.092–0.102 long by 0.061–0.071 broad. The embryo is 0.029–0.031 long by 0.022–0.026 broad and bears six embryonic hooks measuring about 0.010–0.012 long.

Host: Scapanus townsendi Bachmann.

Habitat: Small intestine.

Locality: Stevenson, Washington.

Type specimens: Slides of the type and paratype specimens have been deposited in the helminthological collection of the U. S. National Museum, number 47,599.

Hymenolepis olsoni n. sp. can be separated from most of the species of the genus by the number, size, and shape of the hooks and the relative size and morphology of the egg. The new species can be separated from certain more closely related mammalian forms by the following characters:

Hymenolepis scalopi Schultz, 1939 (parasitic in moles) has an unarmed rostellum which is relatively shorter (0.078–0.117 mm.); the cirrus pouch is proportionately shorter and broader (0.125 × 0.045 mm.); and the eggs are fairly smaller (0.057–0.065 mm.).

Hymenolepis peipingensis Hsu, 1935 (parasitic in moles) attains a considerably greater length (750+ mm.); the rostellum is unarmed; and the testes are arranged in a straight line.

Hymenolepis bacillaris (Goeze, 1782) (parasitic in moles) has a larger number of hooks (36) which are of quite a different shape; the cirrus pouch is smaller (0.120 mm.); and the eggs are slightly smaller (0.071–0.081 × 0.058 mm.).

Hymenolepis petrodomi Baer, 1933 (parasitic in insectivores) is considerably smaller in over-all size (20 × 0.95 mm.); the rostellum is smaller (0.096 × 0.020 mm.); the hooks are not variable in number (10) and are quite differently shaped; and the testes are in a straight line.

Hymenolepis erinacei (Gmelin, 1790) Blanchard, 1891 (parasitic in insectivores) has a larger scolex (0.430 mm.) and a longer rostellum (0.300 mm.) which is unarmed in the adult worm; the testes are arranged in a straight line; and the eggs are much smaller (0.040 mm.).

Hymenolepis scutigera (Dujardin, 1845) Meggitt, 1924 (parasitic in insectivores) is considerably smaller (4–6 × 0.24 mm.); the hooks are not variable in number (10), about twice as large (0.033–0.040 mm.), and are of a different shape; the testes are arranged in a straight line; and the eggs are much smaller (0.042 mm.).

Of the eleven specimens of Townsend's mole examined, only two of the nine collected near Stevenson, Washington, harbored one and five specimens of *Hymeno-*

lepis olsoni n. sp. Two moles taken near Oakville, Washington, were free of this parasite. It is hoped that at a later date more complete data can be presented regarding the incidence of this and other helminths of northwestern moles.

SUMMARY

Two new species of *Hymenolepis* from northwestern mammals are described. Their similarities to other closely related mammalian species are indicated, and notes on their distribution and incidence are recorded.

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EXPLANATION OF THE PLATE

PLATE I

FIG. 1. *Hymenolepis oregonensis* n.sp., scolex.
FIG. 2. *H. oregonensis* n.sp., hook.
FIG. 3. *H. oregonensis* n.sp., proglottid showing fully developed male organs.
FIG. 4. *H. oregonensis* n.sp., proglottid showing fully developed female organs.
FIG. 5. *H. oregonensis* n.sp., egg.

EXPLANATION OF THE PLATE

PLATE II

FIG. 6. *Hymenolepis olsoni* n.sp., scolex.
FIG. 7. *H. olsoni* n.sp., hook.
FIG. 8. *H. olsoni* n.sp., mature proglottid.
FIG. 9. *H. olsoni* n.sp., egg.

PLATE I

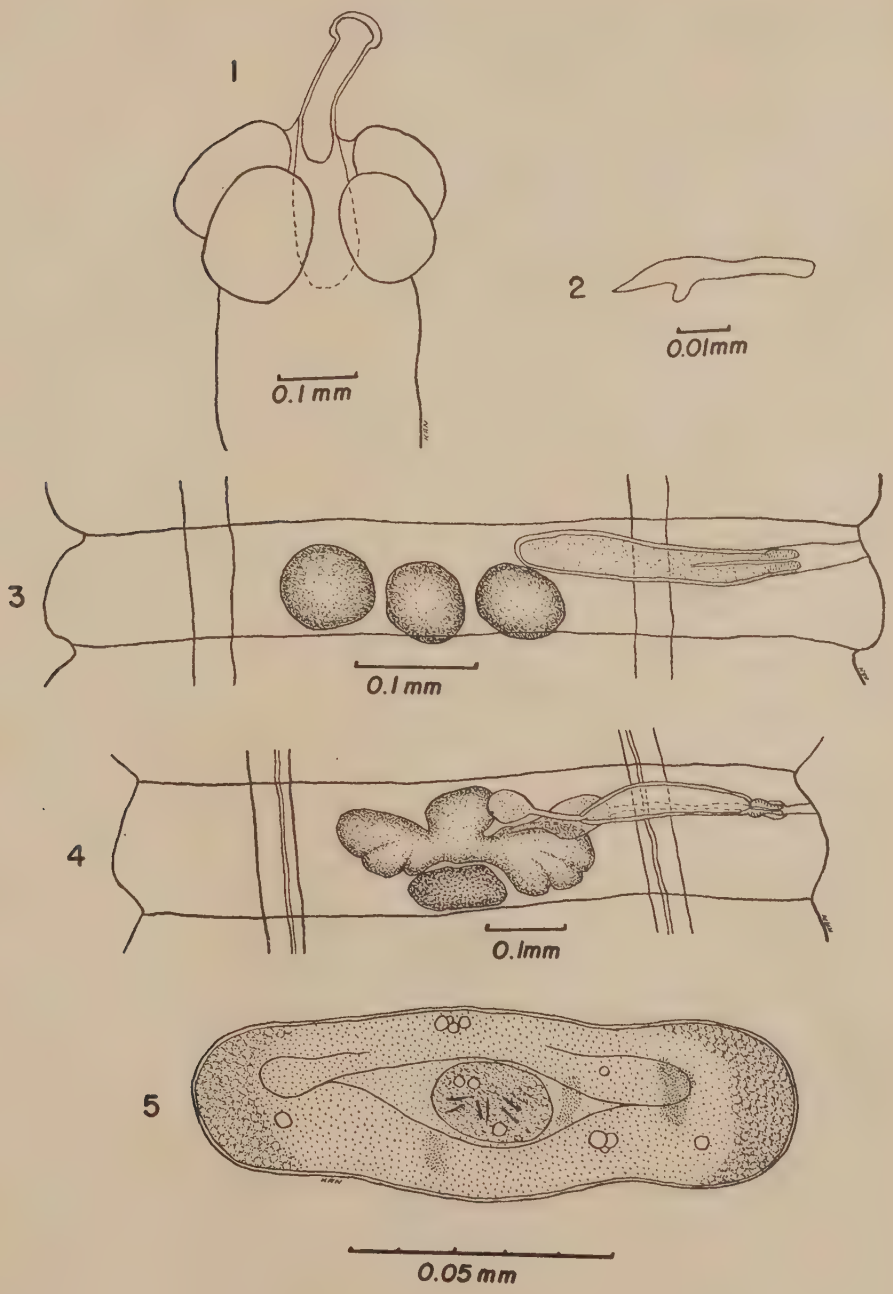
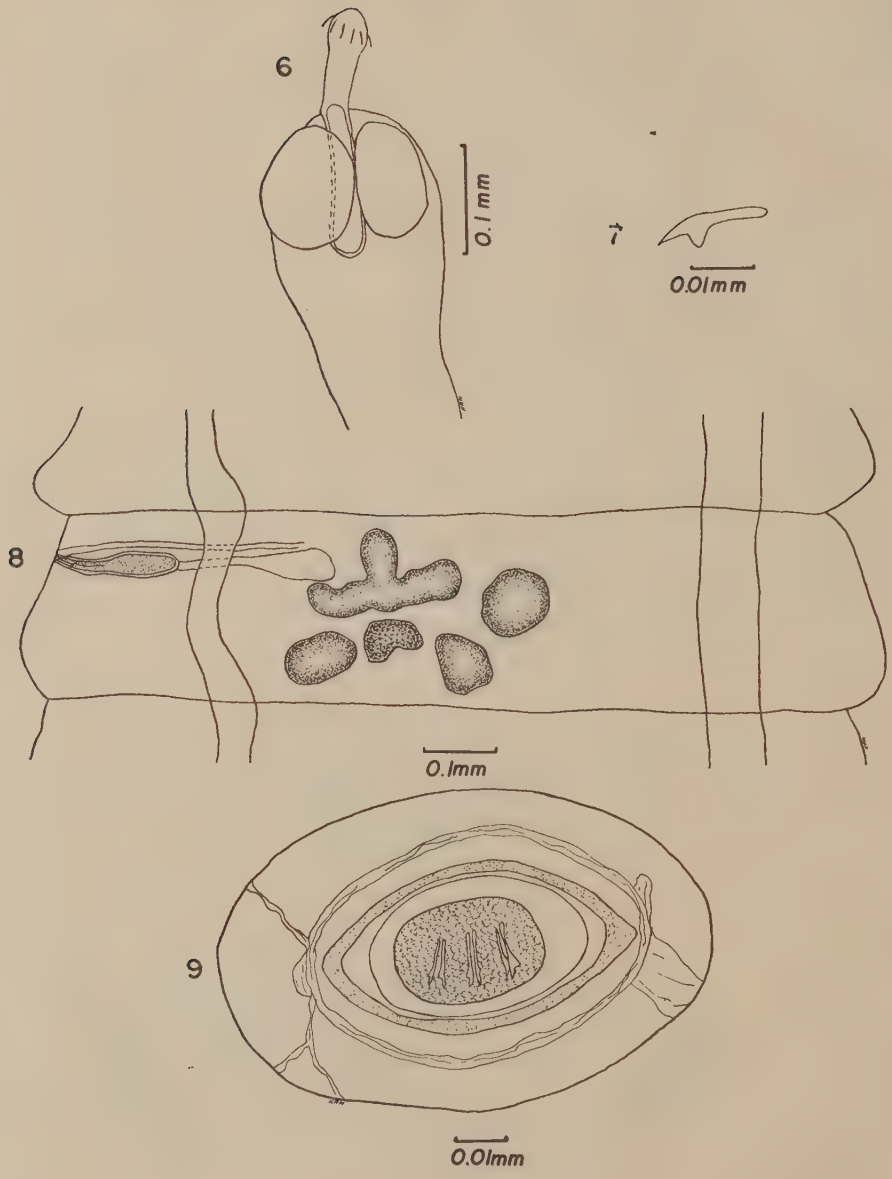


PLATE II



STUDIES ON THE HELMINTH FAUNA OF ALASKA. XI. HELMINTH PARASITES OF MICROTINE RODENTS—TAXONOMIC CONSIDERATIONS

ROBERT RAUSCH*

A survey of the helminth parasites of Alaskan rodents has been carried on by the writer since the winter of 1948–49, as a continuation of an investigation begun in the central United States in 1942. Results of this work indicate that, despite the size of the Territory of Alaska, the survey is complete enough to furnish a reasonable knowledge of the species of helminths parasitic in microtine rodents in Alaska. Information derived from previous investigations serves as a standard against which the completeness of the present work may be judged. In the writer's opinion, the continuation of this work on a survey basis is impractical, and it is the purpose of this paper to report qualitatively the results obtained to date. Main emphasis has been placed here on the taxonomic consideration of the helminth species collected. Ecological and zoogeographical observations will be presented in another publication.

Most of the rodents autopsied in connection with this investigation were collected in Alaska. The material was supplemented whenever possible by rodents from northern Canada and Eurasia.

Only rodents of the subfamily MICROTINAE are considered. A total of 2078 individuals of 26 species and subspecies, representing five genera, has been examined. These comprise the lemmings *(Dicrostonyx* and *Lemmus),* the bog lemmings *(Synaptomys),* the red-backed voles *(Clethrionomys),* and voles of the genus *Microtus.* Nearly all host identifications have been made by the writer, mainly through comparisons with specimens in the collections of the U. S. National Museum, the U. S. Biological Survey, and others. Most of the material from Canada and Eurasia was identified before it came into the hands of the writer.

North American rodents considered in this investigation are listed in Table 1, with brief notations on localities from which they were collected. The map of northern North America (Fig. 1) shows specific collection localities.

The helminthological investigation has been approached from the standpoint of host relationships and distribution, in order to take advantage of obvious faunal relationships and zoogeographical knowledge. Studies of this type are complicated in boreal regions by a tendency toward circumpolar faunal uniformity. Some rodents considered herein are clearly of circumpolar distribution; others are of rather indefinite status, since possible affinities with Palearctic forms have not been elucidated; a few species are Nearctic in their distribution, or at least they have no closely-related Eurasian counterparts. The mouse-like rodents of the subfamily MICROTINAE form a homogeneous group when considered in the light of their immunophysiological relationships with the helminths parasitizing them, as exemplified by little development of host-parasite specificities. These points will be discussed, more appropriately, in another publication.

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TABLE 1.—*Microtine Rodents Examined for Parasites from Arctic and High Boreal Regions of North America*

No.	Name	Number examined	Source
1	*Dicrostonyx groenlandicus groenlandicus* (Traill)	3	Smith Sound, Greenland
2	*D. groenlandicus rubricatus* (Richardson)	30	Arctic Alaska: Wainwright, Point Barrow, Lake Schrader, central Brooks Range
3	*D. groenlandicus richardsoni* Merriam	1	Churchill, Manitoba
4	*D. groenlandicus exsul*[1] Allen	3	St. Lawrence Island
5	*D. groenlandicus* ssp.	5	Prince Patrick Island (Canadian western Arctic)
6	*D. hudsonius* (Pallas)	4	Chimo, Ungava
7	*Synaptomys borealis dalli* Merriam	1	Extreme northwestern British Columbia
8	*Lemmus trimucronatus trimucronatus* (Richardson)	7	Melville Peninsula and Chesterfield Inlet, Canada
9	*L. trimucronatus alascensis* (Merriam)	216	Arctic Alaska: Wainwright, Point Lay, Point Barrow, Lake Schrader, central Brooks Range
10	*L. trimucronatus harroldi* (Swarth)	85	Nunivak Island
11	*L. nigripes* True	1	St. George Island, Pribilofs
12	*Clethrionomys rutilus dawsoni* (Merriam)	240	Alaska: north as far as Umiat, on Colville River, and Lake Schrader—over most of Territory
13	*C. rutilus albiventer*[2] Hall and Gilmore	3	St. Lawrence Island
14	*C. wrangeli* Bailey	11	Southeastern Alaska (Juneau)
15	*Microtus oeconomus operarius* Nelson	324	Western Alaska: North as far as Point Lay, on Arctic Coast
16	*M. oeconomus macfarlani* Merriam	203	Eastern ⅔ of Territory: north as far as Umiat, on Colville River, and Lake Schrader
17	*M. oeconomus innuitus* Merriam[3]	585	St. Lawrence Island
18	*M. oeconomus kadiacensis* Merriam	9	Kodiak Island
19	*M. oeconomus yakutatensis* Merriam	4	Southeastern Alaska: Haines and Valdez
20	*M. oeconomus* ssp.[4]	2	Alaska Peninsula (Cold Bay)
21	*M. pennsylvanicus drumondii* (Audubon and Bachman)	20	Eastern Alaska; Fort Rae, District of Mackenzie
22	*M. longicaudus vellerosus* Allen	6	Extreme northwestern British Columbia
23	*M. longicaudus littoralis* Swarth	9	Southeastern Alaska (Juneau)
24	*M. miurus oreas* Osgood	6	McKinley Park
25	*M. miurus paneaki* Rausch	300	Arctic Alaska: Anaktuvuk Pass region of Brooks Range; Umiat on Colville River; Arctic Village; Lake Schrader
26	*M. miurus* ssp.	1	Talkeetna Mountains, near Anchorage

[1] Hitherto known as *D. exsul* Allen, the writer considers this but a subspecies of *D. groenlandicus*. The study of pertinent material has failed to disclose significant structural differences. According to Ognev (1948; pp. 474–510), *D. groenlandicus* should be considered a subspecies of the Palearctic *D. torquatus* Pallas. A full discussion of this problem is presented elsewhere.

[2] Recent work (Rausch, 1950) has proved the red-backed vole of the Alaskan mainland conspecific with the Palearctic *C. rutilus*. The study of St. Lawrence Island material has failed to disclose structural or other characters of specific value. *C. albiventer* Hall and Gilmore is consequently treated here as a subspecies of *C. rutilus*. Full details will be presented in another publication.

[3] Of these, 493 animals were examined for *Echinococcus* larvae only.

[4] According to recent nomenclatural changes, the specimens of *Microtus oeconomus* from Point Lay should be designated as *Microtus oeconomus gilmorei* Setzer, 1952, (Proc. Biol. Soc. Wash. 65: 75–76), and the Alaskan specimens of *Microtus pennslyvanicus* probably are referable to the form *Microtus pennsylvanicus tananaensis* Baker, 1951, (U. Kans. Publ. Mus. Nat. Hist. 5: 107–108).

In order to simplify reference to the literature, in some cases a brief consideration of the helminth genus is presented before the species are discussed. Detailed morphological data are not included except where it is thought necessary for clarification of a taxonomic problem.

I. CESTODA

Genus *Paranoplocephala* Lühe, 1910

Important among the helminth parasites of North American microtine rodents are cestodes of the genus *Paranoplocephala*. Material obtained during the course of this investigation has been adequate in amount to permit restudy of certain members of the genus. This undertaking has been profitable, since a better concept of speciation within the genus has been attained.

FIG. 1. Localities from which microtine rodents were examined for helminth parasites. Rodents listed in Table 1 were collected from localities represented by solid symbols. Hollow symbols represent collections of rodents whose parasites were studied but not considered specifically in this paper.

Douthitt (1915) was the first to study vole cestodes in the United States. He described two species of *Paranoplocephala*—*P. infrequens* (Douthitt, 1915) and *P. variabilis* (Douthitt, 1915). Baer (1927), in his monograph of the ANOPLOCEPHALIDAE, considered *P. variabilis* identical with *P. infrequens*. An attempt by Rausch and Schiller (1949a) to clarify the status of certain vole parasites resulted in the discovery that *P. infrequens* was incorrectly characterized, and further that

P. infrequens and *P. variabilis* are morphologically distinct. Rausch and Schiller synonomized *P. troeschi* Rausch, 1946, with *P. infrequens,* and at the same time elevated Douthitt's variety, *P. variabilis borealis,* to full specific rank. Baer (1949) reviewed the genus and redescribed *P. isomydis* (Setti, 1892). He also separated the genus into two groups on the basis of the presence or absence of an external seminal vesicle. Voge, in a journal published for 1948, but which appeared after the papers by Rausch and Schiller and by Baer had been published in 1949, described *P. kirbyi* Voge, 1948. *P. omphalodes* (Hermann, 1783) has been recorded twice by North American workers (Harkema, 1946; Rausch, 1951). The most recent addition to the group is *P. neofibrinus* Rausch, 1952. Before the Alaskan species of *Paranoplocephala* are discussed, it is necessary to review some of the foregoing records.

To the time of this writing, *P. infrequens, P. variabilis, P. borealis,* and the recently described *P. neofibrinus* have been considered valid species. Although mentioned earlier in another publication (Rausch, 1952), it is necessary to point out here that *P. kirbyi* is a synonym of *Andrya macrocephala* Douthitt, 1915. The description of *P. kirbyi,* from *Microtus californicus,* was completed before the publication of a study on variation in *A. macrocephala* by Rausch and Schiller (1949b). The examination of type and paratype material, by the present writer, disclosed that these cestodes agree in all morphological details with *A. macrocephala.* The uterus type as portrayed by Voge (1948; Fig. 2) is correct for *Paranoplocephala.* Since the present writer's examination of the material failed to disclose anything but the typical reticulate uterus of *Andrya,* it is assumed that this structure was not correctly interpreted. With the exception of this character, nothing in the description of *P. kirbyi* serves to differentiate it from *A. macrocephala.* It is to be noted that Voge's statement (page 302) could hardly apply to uterus formation in *Paranoplocephala:* "In more posterior mature segments the ends of the uterine tubes become forked and the main tubes give off side branches." The uterus in *Paranoplocephala* characteristically develops through the formation of anterior and posterior sacculations from the simple, transverse tube which comprises the uterus in the mature segments. Except for *P. omphalodes,* discussed below, all species of *Paranoplocephala* recorded from North American hosts possess anteriorly attenuated, more or less wedge-shaped strobilae.

Paranoplocephala infrequens (Douthitt, 1915)

Paranoplocephala infrequens is a common cestode parasite of voles in the United States, and is adapted to existence in the cecum of these animals. It is observed frequently in microtine rodents in Alaska, where abundant material has been obtained. This species was studied in detail by Rausch and Schiller (1949a), and comparisons made by them indicated that the Eurasian *P. brevis* Kirschenblatt, 1938, is a closely related, if not identical, form. Material obtained in connection with the present study allows some elaboration of the earlier work, although *P. infrequens* is a well characterized species. The writer has studied cestodes of this species collected from various hosts over much of North America. The geographic origin of this material can be outlined briefly as follows: From the Mogollon Mountains, Arizona, north to the Arctic Coast of Alaska; from Long Island, New York, west to the Teton Mountains of Wyoming. Specimens have been obtained from various

points lying within these extremes. There is, however, a lack of material from southern and eastern Canada.

Material collected over the United States, nearly all from *Microtus* spp. is relatively uniform. Strobila length ranges from 4 to 11.5 mm. (fully "adult" specimens, with gravid segments and evidence of terminal segment loss). Most of these cestodes range from 5 to 7 mm. in length (average: 6 mm.). The strobila has a characteristic wedge-shape, with the greatest width, usually about 2 mm., attained at the posterior end. The internal detail is essentially as described (Rausch and Schiller, 1949b), but degree of variation in egg size may differ considerably from one locality to another.

About 50 specimens of *P. infrequens* from various hosts and localities within Alaska were stained and mounted for study. It was found that the Alaskan specimens correspond closely in morphological details to those collected much farther south. The average strobila size may be larger, but this does not appear to be

TABLE 2.—*Measurements of* Paranoplocephala infrequens *(Douthitt, 1915) from North American Hosts*

Host	Locality	Strobila length (in mm.)	Greatest strobila width (mm.)	Segment number	Egg size (in microns)	
					Range	Average
Microtus pennsylvanicus pennsylvanicus (Ord)	Long Island, New York	6.5	2	45	32–35 × 20–30	33 × 26
M. pennsylvanicus pennsylvanicus (Ord)	Ohio	5.5–6	2	41–44	33–42 × 23–30	36 × 29
M. pennsylvanicus pennsylvanicus (Ord)	Michigan	5–7.5	2–2.5	39–48	42–56 × 23–49	47 × 31
M. pennsylvanicus pennsylvanicus (Ord)	Wisconsin	5–7	1.2–2	30–31	36–56 × 29–52	48 × 41
M. pennsylvanicus drummondii (Aububon and Bachman)	Skwentna River, Alaska	5.7–6.5	1.5–2	31–33	33–39 × 26–29	35 × 28
M. pennsylvanicus modestus (Baird)	Wyoming	5–6	1.2–1.5	28–32	35–46 × 27–43	42 × 37
M. montanus nanus Merriam	Wyoming	5–7	2–3	29–36	36–49 × 26–42	45 × 35
M. richardsoni macropus Merriam	Wyoming	4–7	2.5	26–42	35–51 × 35–40	45 × 38
M. mogollonensis Mearns	Arizona	6–7	1.7–2	32–35	39–46 × 29–36	43 × 31
M. miurus paneaki Rausch	Anaktuvuk Pass, Brooks Range, Alaska	7–9	3.1–4	41–42	36–49 × 29–46	44 × 36
M. miurus paneaki Rausch	Lake Schrader, Alaska	7–7.5	2–2.7	41–42	43–51 × 37–50	46 × 42
M. oeconomus macfarlani Merriam	Anaktuvuk Pass, Brooks Range, Alaska	6–7.5	2–3	32–42	39–66 × 36–56	55 × 46

significant. There is, however, the tendency toward greater local variation in egg size. Cirrus sac size is variable, as is testes number. Testes distribution, on the other hand, appears to be a rather important specific character if minor differences are not emphasized.

In order to compare cestodes of this species from various hosts and from various parts of North America, important measurements and other details have been presented in Table 2.

It is possible to draw the following conclusions from present knowledge of *P. infrequens:*

1. Strobila size is not related to host-species occurrence.
2. Morphological differences cannot be correlated with host-species occurrence.
3. Egg size is highly variable in *P. infrequens;* both size-range and average size may differ considerably from one locality to another.

4. Although specimens from Alaska seem more variable locally and average somewhat larger in size, there is no apparent cline formation.

Paranoplocephala borealis (Douthitt, 1915)

When one studies a good series of cestodes corresponding morphologically to the description of *P. borealis,* it becomes apparent that only specimens of a uniform state of development are included. Douthitt (1915) was of the opinion that a graded variation from south to north existed, involving strobila size and certain internal structures. In this regard he stated (1915; p. 23): "As one passes northward, however, it is found that the individuals grow steadily smaller, both in bulk and number of proglottids. In anatomical features only one difference was discerned: the testes become very regularly fewer. . . . The most conspicuous difference between the worms from the different localities is in size; those from the north being only about half the length and breadth of those from the south." Rausch and Schiller (1949a) did not observe morphological intergradation of *P. variabilis* and *P. borealis* in their material from the Great Lakes region. It was thought by them that two distinct species were involved, on the basis of characters considered at that time to have specific value. Douthitt, on the other hand, stated, in reference to his table (page 24), that ". . . while there is a considerable difference between the extremes, the intergradations are sufficiently regular to destroy the validity of these differences as specific characteristics." Except for a small amount of Douthitt's original material, mostly in the form of serial sections, the writer has not examined any specimens from the type host, *Geomys bursarius* Shaw, in which *P. borealis* is reported to occur in large numbers per individual animal.

In Alaska, particularly in the central Brooks Range and in the south along the Skwentna River, a good series of the *P. borealis* type was collected. It is evident from this material that specimens classified as *P. borealis* represent young cestodes in which fully-formed eggs are just appearing. This impression is supported by the persistence of the original terminal segment until the strobila in some cases has reached a length as great as 8 mm., and the loss of the terminal segment alone in strobilae up to 15 mm. in length. Some strobilae, on the other hand, may lose terminal segments by the time a length of 5 mm. is attained, there being no consistency in this feature.

Specimens typical of *P. borealis* have been obtained by the writer from the following hosts and localities, in addition to the Alaskan material: *Microtus p. pennsylvanicus* Ord, from Wisconsin, Ohio, and southern Illinois; *M. ochrogaster* (Wagner), from Nebraska; *Synaptomys cooperi* Baird, from southern Illinois; *Thomomys talpoides tenellus* Goldman, from Jackson Hole, Wyoming; *Microtus* sp. from Oregon. There is striking uniformity in these specimens, despite their wide geographic distribution.

There are two anatomical details, however, which require further mention if the identity of the Alaskan cestodes is to be established; these are cirrus spination and egg size.

Douthitt (1915; p. 21), in regard to *P. variabilis,* stated, "The cirrus is not spiny." This was verified by Rausch and Schiller (1949a) for both *P. variabilis* and *P. borealis.* The study of Alaskan material, however, has disclosed that the presence of cirrus spines cannot be doubted. Since cirrus spination is often used as a specific

character in cestode differentiation, it is important to evaluate properly this disagreement of observations. The study of all available material of the *"borealis"* type has shown without doubt that Alaskan specimens are conspecific with those collected farther south. Further, the fact that cirrus spines are sometimes not visible on the Alaskan specimens indicates some kind of irregularity in this character, either actual or resulting from the treatment of the material. After consideration of all possibilities, there is only one tenable conclusion: cirrus spines are often lost through partial degeneration of the cestodes when they are not removed immediately after the death of the host. This conclusion is supported by the statement by Joyeux and Baer (1936), in reference to another species of cestode, *Hymenolepis horrida* (von Linstow, 1901), that "Le cirre est armé sur toute sa longueur de petits crochets qui peuvent disparâitre dans les échantillons macérés." Schiller's (1952b) study of the same species of *Hymenolepis* has not only supported Baer's work, but has also disclosed much variation in size of cirrus spines. Various factors contribute to delayed autopsy of animals collected for study. Rate of degeneration of host tissue, with attendant maceration of cestodes in the intestinal tract, depends upon weather and upon host species. The usually cool temperatures of northern Alaska, coupled with the fact that much of the autopsy work was done daily in the field at the time of collection of the mammals seems to have contributed to a greater suitability of the resulting material for study.

That egg size is variable cannot be doubted, although the lessened value of this character in connection with species differentiation contributes further to the already numerous difficulties associated with the identification of anoplocephalid cestodes. Again, as with *Andrya macrocephala,* there is no graded variation. Egg size differs from one locality to another with no apparent regularity. There is a tendency for Alaskan cestodes to show a greater range in egg size, and a greater average measurement, than do specimens from the central United States.

The additional data on *P. borealis* obtained from the study of the Alaskan material make it clear, in the writer's opinion, that this name refers only to a stage in the growth of *P. variabilis.* The name *Paranoplocephala borealis* (Douthitt, 1915), consequently becomes a synonym of *P. variabilis* (Douthitt, 1915), which is discussed briefly below.

Paranoplocephala variabilis (Douthitt, 1915)

Since the foregoing discussion of *P. borealis* applies directly here, only a few additional remarks on *P. variabilis* are necessary. It is evident that *P. variabilis* is a species of wide distribution in North America. It occurs from the Arctic Coast of Alaska south to at least the Lake States, and over this range it parasitizes several species of rodents. The possibility of its occurrence in Eurasia should not be overlooked, although there is at present no evidence that it has Holarctic distribution.

On the basis of gross appearance, *P. variabilis* is readily differentiated by its anteriorly attenuated, elongate strobila from other species of *Paranoplocephala* occurring in voles (see Rausch and Schiller, 1949a; Figs. 1 and 4). As mentioned above, *P. variabilis* exhibits considerable morphological variation, much of which is associated with the age of the individual cestode. In common with *P. infrequens,* it exhibits a rather wide range of egg sizes. The eggs of Alaskan specimens ranged

from 32 to 42 μ long by 30 to 40 μ wide, while those from the United States usually possess smaller eggs. Douthitt (1915; p. 22), for specimens from *Geomys,* gave the egg size as 30 to 35 μ. The specimens studied by Rausch and Schiller (1949a) had smaller eggs, from 26 to 33 μ long by 20 to 26 μ wide. Egg size is too variable to have specific value *per se,* particularly since such variations are local, and do not show any gradation in relation to geographic distribution.

Paranoplocephala omphalodes (Hermann, 1783)

Paranoplocephala omphalodes, the type species of the genus, is a well-known vole parasite in Europe. The only authentic record of its occurrence in North America is that of Rausch (1951). According to the writer's observations, *P. omphalodes* is limited to arctic Alaska in its North American distribution. It is a rather common parasite of the narrow-skulled vole, *Microtus miurus paneaki,* in the central Brooks Range.

The Alaskan specimens of *P. omphalodes* are readily differentiated from the other North American members of the genus on the basis of strobila form. It has a greatly elongate, *Andrya*-like strobila, with a prominent scolex. Although it superficially resembles *Andrya macrocephala,* it differs in segment shape, having a relatively high length/width ratio.

Although listed as a separate species in Baer's (1927) monograph of the family Anoplocephalidae, *P. blanchardi* (Moniez, 1891) was considered identical with *P. omphalodes* by Joyeux and Baer (1936). In a later publication (Baer, 1949), the situation is further discussed: "La différence de taille entre ces deux espèces, à première vue si marquée, disparait lorsqu'on possède on matériel suffisant conservé à divers degrés de macération. Les échantillons très contractés atteignent une largeur de 5 mm. tandis que ceux qui sont étirés et macérés, atteignent à peine 1 mm. Chez les deux espèces, la poche du cirre renferme un cirre armé et les oeufs sont munis d'un appareil piriforme. Dans les deux cas, également, on trouve une alternance très irrégulière des pores sexuels qui ne sont jamais complètement unilatéraux."

The conclusion that *P. blanchardi* is conspecific with *P. omphalodes* requires the recognition (in Europe) of two "forms" of *P. omphalodes,* with strobila lengths from "20 à 40 mm. ou 100 à 215 mm.", and with genital pores either "unilatéraux ou irrégulièrement alternes." In North America such a situation is not evident. All material examined by the writer compares well with the description of *P. omphalodes* given by Baer in his earlier publication (1927). Material was adequate in amount, and condition, to allow thorough study.

Since the writer's material is the first of this species to be studied from North American hosts, a short diagnosis of the species is included herewith:

Paranoplocephala omphalodes (Hermann, 1783)

(Figs. 2–3)

Diagnosis: Strobila length 150 to 195 mm.; maximum width, attained in postmature segments, 4 mm. Strobila ribbon-like, with serrate margins. Segment-number up to 350. Mature segments occur in narrow, attenuated strobila-section immediately following scolex; length/width ratio of mature segments about 1 : 10. Length/width ratio of early gravid segments 1 : 5; of late gravid segments 1 : 2.7. Scolex well developed, distinctly set off from neck. Genital pores irregularly alternate, situated in anterior third of segment margin. Testes spherical, 51 to 61 in number, all situated in aporal half of segment. Most porally situated testes rarely in

contact with aporal limit of ovary. Cirrus sac about 250 by 70 μ in mature segments; cirrus spinose. Internal seminal vesicle present. Vagina postero-ventral to cirrus sac. Strongly-lobed ovary situated in poral half of segment. Vitelline gland near middle of ovary, usually somewhat poral. Tubular uterus present in mature segments; uterine growth takes place through development of regular anterior and posterior sacculations. Gravid uterus nearly fills terminal segments. Eggs measure 40–52 μ long by 32–43 μ wide (average: 43×35 μ).

North American host: Microtus miurus paneaki Rausch.

Distribution: Tulugak Lake, Brooks Range, Arctic Alaska. (Lat. 68° 20′ N., Long. 151° 26′ W.)

Habitat: Small intestine of host.

A slide bearing a complete specimen has been deposited in the Helminthological Collection of the U. S. National Museum, Slide No. 47803.

Paranoplocephala lemmi n. sp.

At Point Barrow, Alaska, during the early spring of 1949, the writer collected from the brown lemming a few cestodes closely related to *P. infrequens*. In a preliminary report of lemming parasites (Rausch, 1950), these specimens were designated as *P. infrequens;* however, subsequent study of this and additional material has revealed that this species is distinct. It is described herewith as

Paranoplocephala lemmi n. sp.

(Figs. 4–7)

Diagnosis: Strobila length 10 to 20 mm.; maximum width, attained near posterior end, 3 to 7 mm. Strobila wedge-shaped, often with slight narrowing of terminal gravid segments. Margins serrate; neck short. Length/width ratio of mature segments as great as 1 : 10; little increase in length of gravid segments. Segments number from 68 to 79. Scolex from 1 to 1.6 mm. in width, very distinct from neck. Suckers powerful. Ventral longitudinal excretory canals about 30 μ in diameter; dorsal canals, lateral to latter, about 20 μ in diameter. Genital pores unilateral; dextral; situated near middle of segment margin. Genital canals dorsal to longitudinal excretory canals. Testes subspherical, 56 to 73 in number (average: 67), about 70×80 μ in mature segments. Testes extend from about middle of vitelline gland to aporal longitudinal ventral excretory canal, or just beyond it, and in earlier mature segments are situated in almost single row across segment; antero-posterior distribution much restricted. Cirrus sac strongly developed; pyriform, from 300 to 720 μ long by 100 to 180 μ wide; cirrus slender, provided with inconspicuous spines. Internal and external seminal vesicles highly developed. Vagina ventral and posterior to cirrus sac. Seminal receptacle well developed. Vitelline gland, variable in shape but usually lobed, situated near center of ovary. Ovary highly lobed, situated in poral half of segment. Tubular uterus in mature segments does not pass laterally beyond longitudinal ventral excretory canals; it does not extend aporally as far as testes. Uterine sacculations gradual in development. Gravid segments not filled by uterus beyond longitudinal excretory canals. Eggs variable in size, nearly spherical, from 51 to 67 μ long by 41 to 56 μ wide (average: 51×58 μ). Pyriform apparatus well developed; embryo only about 15 μ long.

Type host: Brown lemming, *Lemmus trimucronatus alascensis* Merriam. Additional hosts: *L. t. trimucronatus* (Richardson)—Melville Peninsula and Chesterfield Inlet, N. W. T.; *L. t. harroldi* (Swarth)—Nunivak Island.

Type locality: Point Barrow, Alaska.

Habitat: Cecum of host.

Type: A whole-mount, containing type and paratype specimens, has been deposited in the Helminthological Collection of the U. S. National Museum, Slide No. 37355.

Discussion: P. lemmi n. sp. is closely related to *P. infrequens*, which it resembles in regard to habitat, strobila shape, and morphological details. It differs clearly from this species in strobila size and segment number, in egg size, size of cirrus sac, testes number and distribution, and in character of the uterus. Host occurrence may be diagnostic, according to present knowledge. *P. lemmi* n. sp. may also be limited to the North American arctic in its geographic distribution.

Genus *Andrya* Railliet, 1893

The species of *Andrya,* so important in the parasite-fauna of microtine rodents, often present unusual difficulties in identification. There is a lack of consistent morphological characters, and variation may be so great as to exceed any but the broadest species definitions. Only four species are now recognized in North America (viz., *A. primordialis* Douthitt, 1915; *A. macrocephala* Douthitt, 1915; *A. neotomae* Voge, 1946; *A. sciuri* Rausch, 1947). The literature has been reviewed in recent publications (Rausch, 1947; 1948; Rausch and Schiller, 1949b). Three species of *Andrya,* one of which is described as new, are considered in the present paper.

Andrya macrocephala Douthitt, 1915

Cestodes regarded as *A. macrocephala* were collected, mainly from species of *Microtus,* over much of the Territory of Alaska. Variation in morphological details was noted, and this variation exceeded the limits established earlier from the study of material obtained in the United States (Rausch and Schiller, 1949b). The degree of variation in certain characters was found to differ from one locality to another in Alaska, but there was no evidence anywhere of variation of a continuously-graded nature.

In the earlier work by Rausch and Schiller (1949b), it was concluded that egg size, in conjunction with any other characters of apparent taxonomic value (such as testes distribution, relative size of cirrus sac, etc.), was particularly important in the differentiation of species of *Andrya.* It is now possible to evaluate this character from a much broader standpoint insofar as host-species occurrence and geographical distribution are concerned. For the present study, specimens of *A. macrocephala* collected from New York west to California and Washington, and from Mexico City north to central Alaska and St. Lawrence Island, in the Bering Sea, were available. Over the southern part of North America, specimens exhibited no appreciable disagreement with the diagnosis of *A. macrocephala* as established by Rausch and Schiller (1949b).

Although the cestodes showed considerable uniformity in regard to general morphological details, variation in egg size was especially marked in the Alaskan specimens. A cestode collected about 100 miles southeast of Fairbanks was typical of *A. macrocephala* in every detail, with an average egg size of $33 \times 28\mu$. Specimens from the same host *(Microtus oeconomus macfarlani)* collected farther north, at Big Delta, disclosed a larger average egg size, $39 \times 31\ \mu$, but otherwise showed no disagreement with *A. macrocephala.* The greatest extremes in egg size were noted in cestodes collected from rodents on St. Lawrence Island. The eggs of specimens from St. Lawrence Island voles, *Microtus oeconomus innuitus,* ranged in size from 36 to 48 μ long by 27 to 38 μ wide. A good series of cestodes was also obtained from ground squirrels, *Citellus parryii lyratus* Hall and Gilmore, and in these the greatest extremes in egg size to be recorded in Alaska were seen. These eggs ranged in size from 40 to 51 μ long by 30 to 41 μ wide. The study of these specimens failed to disclose other morphological differences. If knowledge of cestode speciation were more complete, it might in some cases be expedient to utilize a subspecific designation.

Two generalizations appear to derive from these observations on *A. macroceph-*

ala: (1) Great variation in egg size and egg shape (ovoid to spherical) must be accepted; (2) There is no recognized correlation between morphological variation in *A. macrocephala* and its host-species occurrence.

It becomes obvious that a broad view of this species must be taken, and great care must be exercised in the description of new species of *Andrya,* unless distinct morphological differences are evident.

Andrya primordialis Douthitt, 1915

The status of *Andrya primordialis* was reviewed to complete the study of Alaskan cestodes of the genus *Andrya.* The examination of all available material has made certain discrepancies obvious in the concept of this species.

Douthitt (1915) described two cestodes, *Andrya primordialis* and *A. communis,* and pointed out (page 10) that "Many of the differences in the two accounts are due probably to differences in the state of contraction in the material." In his monograph of the ANOPLOCEPHALIDAE, Baer (1927) concluded that *A. communis* was identical with *A. primordialis,* and included a revised diagnosis of the latter. Two cestodes from *Tamiasciurus hudsonicus* Erxleben furnished the basis for the description of *A. primordialis,* while *A. communis* was described from more abundant material, consisting of ". . . about 200 fragments in alcohol and 17 slides" (Douthitt, 1915; p. 8), from *Clethrionomys gapperi galei* Vigors. The present writer has been able to secure, of Douthitt's original material, only serially-sectioned specimens. In the case of *A. communis,* nearly all mounted material available to Douthitt was already sectioned when he received it, since he stated that only a single scolex with "a considerable number of proglottids attached" was examined, thus "furnishing an idea of the appearance of the complete worm."

In any event, Douthitt was unable to give egg size for either of his species, but made some observations on the developing uterus. Baer (1927; p. 35) published an egg size of 35 μ for *A. primordialis.* Dr. Baer (personal communication) is not certain of the origin of this figure, but suggested that it might have been derived from *A. primordialis* var. *gundii* Joyeux, 1923. The figure also closely approximates the egg size of *A. macrocephala.* Since there has been no subsequent publication of egg size for *A. primordialis,* the description of the species is incomplete.

A pedunculated prostate gland was described by Douthitt for both *A. primordialis* and *A. communis.* This character alone should differentiate *A. primordialis* from the other North American species (viz., *A. macrocephala* Douthitt, 1915; *A. neotomae* Voge, 1946; *A. sciuri* Rausch, 1947). Of North American species of *Andrya, A. primordialis* is also unique, according to published descriptions, in the possession of unilaterally-arranged genital pores.

For some time the writer has recognized the indefinite status of *A. primordialis.* The examination of several hundred specimens of *Andrya* spp. has failed to disclose any individuals which correspond to this species as described. Individuals possessing unilateral genital pores have not been observed, although a condition approaching this has been noted in certain Rocky Mountain material. The writer has not been able to determine with certainty the presence of a prostate gland, either from his own material or from Douthitt's material such as could be obtained from the U. S. National Museum collection (a total of seven slides; six of these marked *A. primordialis* and one marked *A. communis*—all serial sections).

The lack of any egg production in many strobilae further complicates the problem, since in such cases one of the most important differentiating characters—that of egg size—is lost. Even though the range of egg size may be great, it is often significant when considered in relation to other details.

A limited amount of material which seems clearly to represent the species *A. primordialis* has been studied. A specimen was obtained from one of the type hosts, *Tamiasciurus hudsonicus*, collected in Wyoming. This strobila, about 80 mm. long, showed a normal uterus development in all segments. The eggs measured from 52 to 66 μ long by 40 to 56 μ wide (average: 62×48 μ). The testes, in most segments included between the ventral excretory canals, numbered from 39 to 55. The cirrus sac measured from 108 to 135 μ long, by 52 to 66 μ wide (average: 119×60 μ). The slide containing this cestode has been deposited in the Helminthological Collection of the U. S. National Museum, Slide No. 47801.

Considerable material from *Phenacomys i. intermedius* Merriam, from the same locality as above, was also examined. This agreed closely with the specimen from the squirrel.

Without taking into consideration the prostate gland, egg size in combination with other predominant characters serves to differentiate *A. primordialis* from the other species of the genus, their variability notwithstanding. It should be mentioned that few specimens of *A. neotomae* and *A. scirui* exist in collections, so there is little knowledge of their limits of variation. In addition to the 13 specimens of *A. neotomae* collected by Voge (1948) from *Neotoma fuscipes* ssp., the writer has obtained about 20 specimens from *N. cinerea* Ord, in Oregon. Of *A. sciuri,* only the original material is known. These species appear to be well differentiated.

The writer hesitates to assign any Alaskan specimens of *Andrya* to the species *A. primordialis.* Still, certain cestodes of relatively small size, agreeing with *A. primordialis* in testes distribution, and having no apparent development of the external seminal vesicle, should perhaps be assigned to this species. The possibility of their being immature or aberrant individuals of *A. macrocephala* cannot be overlooked, however, The solution of this problem is dependent upon the availability of adequate material for study—probably best obtainable in the Rocky Mountain region of the western United States.

Andrya arctica n. sp.

In the early spring of 1949, in the course of examination of lemmings (particularly *Dicrostonyx*) in the vicinity of Point Barrow, the writer collected several cestodes of the genus *Andrya* which, by virtue of a small, delicate strobila, relatively large cirrus sac, and large eggs, seemed clearly to represent an undescribed species. Later, when the confused status of *A. primordialis* was realized, and since some variation had been seen by this time in the lemming form, the latter was tentatively assigned to the species *A. primordialis* (see Rausch, 1950; 1951). The similarity of egg sizes, particularly, brought about this conclusion, since the previous experience with *A. macrocephala* had led the writer to consider this character to be relatively stable. However, after a review of the literature and the study of all available material, it is concluded that the lemming cestode is distinct from *A. primordialis.* It is described herewith as new.

Andrya arctica n. sp.

(Figs. 8–9)

Diagnosis: Strobila 50 to 145 mm. long; maximum width, attained near posterior end of strobila, 1 to 2.4 mm. Mature segments often as long as broad, but extreme ratio of length to width may be as great as 1 : 5. Scolex from 215 to 560 μ wide, strongly set off from thin neck; suckers about 80 by 110 μ. Segmental margins serrate. Genital pores irregularly alternate, situated in the posterior third of the segment margin. Cirrus sac strongly developed, from 194 to 352 μ long by 57 to 136 μ wide in mature segments. Cirrus sac often extends across 1/3 of segment width. Cirrus spinose. Internal and external seminal vesicles strongly developed. Testes usually from 40 to 50 in number; about 80 μ in diameter in mature segments. Testes extend, in the extreme, from proximal end of cirrus sac to well beyond aporal longitudinal ventral excretory canal. Testes in some cases not seen farther poral than poral edge of ovary. Entire anterior field of segment occupied by testes. Prostate gland absent. Vagina posterior to cirrus sac; very large seminal receptacle, usually spherical in shape, seen in post-mature segments. This organ often extends well into following segment. Ovary situated in middle of segment, near posterior edge. Vitelline gland, relatively large, situated at posterior margin of segment, near middle; its width may equal as much as 1/4 of mature segment width. Uterine development clearly reticulate; development somewhat asymmetrical, with posterior extension more rapid on aporal side. Terminal segments completely filled with large eggs; excretory canals usually not persistent in gravid segments. Eggs measure (not in a single strobila) from 40 to 72 μ long by 26 to 65 μ wide. The average egg size is about 65 × 50 μ. Normal egg shape ranges from ellipsoid to spherical, within the same segment.

Type host: Dicrostonyx groenlandicus rubricatus (Richardson). Also collected from *D. groenlandicus richardsoni* Merriam—Churchill, Manitoba; *D. groenlandicus* ssp.—Prince Patrick Island; *Lemmus trimucronatus alascensis* (Merriam)—Point Barrow, Alaska; *Clethrionomys rutilus dawsoni* (Merriam)—Umiat, Alaska; *Microtus miurus pancaki* Rausch—Umiat, Alaska.

Type locality: Point Barrow, Alaska.

Type: A slide bearing an entire specimen has been deposited in the Helminthological Collection of the U. S. National Museum, Slide No. 37356.

Discussion: Andry arctica n. sp. clearly belongs in the subgenus *Aprostatandrya* as it was originally defined by Kirschenblatt (1938).[1] This in itself eliminated *A. primordialis* from consideration, its present controversial status notwithstanding. The writer is unwilling to emend the specific diagnosis of *A. primordialis,* beyond the point of egg size discussed above, without clear-cut evidence.

To the best of the writer's knowledge, the subgenus *Aprostatandrya* Kirschenblatt, 1938, contains the following species: Palearctic—*A. africana* Baer, 1933; *A. monodi* Joyeux and Baer, 1930; Nearctic—*A. macrocephala* Douthitt, 1915; *A. neotomae* Voge, 1946; *A. sciuri* Rausch, 1947. In another publication (Rausch and Schiller, 1949b), *A. caucasica* Kirschenblatt, 1938, was provisionally considered identical with *A. macrocephala.* According to the published description, *A. białowienzensis* Sołtys, 1947, possesses a prostate gland and need not be considered here.

A. arctica can be readily differentiated from *A. monodi* and *A. africana* on the basis of testes distribution, as well as relative organ size differences. From *A. neotomae* and *A. sciuri, A. arctica* differs in relative organ size, egg size, and testes distribution. From *A. macrocephala* it differs essentially in egg size and relative size of cirrus sac.

It is possible that adaptation to host species (it is predominantly a parasite of lemmings) and geographical distribution (arctic North America) also have some specific significance. A single specimen of cestode in the writer's collection, from *Lemmus lemmus* L., collected at Enontekis, Finland, possibly should be referred to this species, but the available material does not allow certainty in this. *A. arctica*

[1] Recently, Russian workers have given Kirschenblatt's subgenera full generic standing. The writer does not agree with this concept.

must therefore be considered an helminth restricted in its distribution to the North American Arctic until evidence to the contrary is obtained.

Genus *Catenotaenia* Janicki, 1904

The genus *Catenotaenia* is not well represented in Alaska, either from the standpoint of species or of individuals. Only one species—*C. reggiae* Rausch, 1951—occurs commonly as a parasite of a hoary marmot, *Marmota caligata broweri* Hall and Gilmore, in the arctic Brooks Range region.

Catenotaenia dendritica (Goeze, 1782)

Collected but once from a red-backed vole, *Clethrionomys rutilus dawsoni*, near Anchorage, Alaska, this cestode is a rare parasite of microtine rodents in the Territory. The single specimen obtained was typical. The writer has collected this parasite also from *C. gapperi cascadensis* Booth, from the state of Washington. With possible local exceptions, it is not a common parasite in North American microtine rodents.

Genus *Paruterina* Fuhrmann, 1906

Cestodes of the genus *Paruterina* are, in the adult stage, parasitic in birds. According to present knowledge, *P. candelabraria* (Goeze, 1782) is the only species for which mammals serve as the intermediate host. It is a widely-distributed, circumpolar species (Wolffhügel, 1900), particularly common in Alaska in the snowy owl, *Nyctea scandiaca* (L.).

Paruterina candelabraria (Goeze, 1782)

Although the larval stage of this owl parasite was not actually observed in any of the rodents examined, it was nevertheless a common parasite of the snowy owl along the Arctic Coast of Alaska. The writer found 29 of these owls infected, of 112 individuals autopsied. These birds were all collected along the Arctic Coast from Wainwright to Point Barrow, with the exception of two specimens from St. Lawrence Island. This cestode has been recorded by the writer, also, from the hawk-owl, *Surnia ulula caparoch* (Müller), Richardson's owl, *Aegolius funereus richardsoni* (Bonaparte), and the saw-whet owl, *A. acadicus acadicus* (Gmelin), all from southern Alaska. In view of the life-cycle requirements of this cestode (Rausch, 1949), it is evident that microtine rodents in northern Alaska must serve as its intermediate host.

It was remarked by Rausch (1949) that the cysticercoids of *P. candelabraria* were unknown from natural infections of rodents. The writer has since been advised by Dr. Reino Freeman, Department of Zoology, Southern Illinois University, that he has collected naturally-infected rodents in Minnesota. Dr. Freeman expects to publish the details of his observations.

Genus *Hymenolepis* Weinland, 1858

Cestodes of the genus *Hymenolepis* were poorly represented in the material collected from Alaskan and north Canadian microtine rodents. Only two species, *H. horrida* (von Linstow, 1901) and *H. johnsoni* Schiller, 1952a, have been collected.

Hymenolepis horrida (von Linstow, 1901)

A well-known parasite of Eurasian voles, *Hymenolepis horrida* was first recorded from North American rodents by Kuns and Rausch (1950). The present investigation has shown it to be perhaps the most common and widespread cestode parasite of boreal rodents on the continent. Farther south it is rare, occurring mainly in voles found in sub-alpine or alpine habitats (Rausch, 1951); however, further investigation is required to clarify the characteristics of its occurrence beyond the limits of boreal spruce forest.

The abundant material obtained in connection with this work has been studied by Mr. E. L. Schiller, of this laboratory. His work disclosed the occurrence of rather extreme morphological variation in *H. horrida,* which has necessitated a modification of the species-concept (Schiller, 1952b).

Hymenolepis johnsoni Schiller, 1952

Four specimens of a cestode, recently described by Schiller (1952a) as *Hymenolepis johnsoni,* were obtained from a vole, *Microtus pennsylvanicus drummondii,* trapped at Fort Rae, on Great Slave Lake. No other record of this cestode has been obtained. Since nothing is known of the species beyond the original description, further discussion of it here is not warranted.

Genus *Echinococcus* Rudolphi, 1810

Cestodes of the genus *Echinococcus* are important parasites of canine animals throughout Alaska. Only on St. Lawrence Island is the life-cycle of a cestode of this genus connected with microtine rodents.

Echinococcus sp.

The occurrence of the larval form of *Echinococcus* sp. in voles, *Microtus oeconomus innuitus,* on St. Lawrence Island, was reported by Rausch and Schiller (1951). Subsequent studies on this island have shown further that a red-backed vole, *Clethrionomys rutilus albiventer,* is also an important intermediate host of this cestode. The red-backed vole, however, has not been abundant during the time of these observations (1950–52); only three specimens, two infected, have been obtained.

St. Lawrence Island lies in the Bering Sea just off the Siberian Coast, and there is considerable evidence that the species of *Echinococcus* found there is of Asiatic origin. This hypothesis is particularly tenable when one considers that the *Echinococcus* situation on Bering Island, in the Komandorskii group, appears to be epizootiologically identical with that on St. Lawrence Island. On Bering Island, *C. rutilus* is the intermediate host. In this regard, Barabash-Nikiforov (1938) stated that ". . . almost 50 per cent of these animals are infested with an intermediate stage of *Taenia echynococcus.*" More important were the observations of Afanas'ev (1941), who considered *Evotomys rutilus* (= *C. rutilus*) the only intermediate host of "*Echinococcus granulosus* (Batsch)" on the Island. He also made numerous observations on slaughtered domestic animals (cattle, swine, reindeer), but failed to find any of these infected. Afanas'ev found no trace of this parasite on Mednii Island, and considered it absent here because the red-backed vole also was absent. (". . . po prichine otsutstviia tam ego promezhutochnogo khoziaina—polevki.")

Bering Sea currents unquestionably are such that mammals are transported on floating ice from Siberia to St. Lawrence Island. A full discussion of this point will be included in a later report.

The species of *Echinococcus* occurring on St. Lawrence Island differs at least immunologically from the mainland form, which the writer regards as *E. granulosus* (Batsch, 1786). On the Alaskan mainland, where *Echinococcus* is a common parasite of canine animals, the intermediate hosts are moose and caribou. There is no record of natural rodent infection by the larval form of this cestode on the mainland.

Cross-infection experiments, using mainland material as well as that from the Island, have substantiated field observations relative to peculiarities of intermediate-host occurrence. Observations on a variety of experimental animals (cricetids, murids, sciurids, geomyids) to which infective eggs were administered have supported the conclusion that voles are readily infected by the St. Lawrence Island species, but no success has been achieved in the infection of rodents with mainland material. To date, wild-caught *Microtus californicus* (from California), laboratory-reared *M. pennsylvanicus,* wild-trapped *C. rutilus dawsoni* (Alaskan mainland), a wild-caught muskrat, *Ondatra zibethica* ssp. (Alaskan mainland), and wild-trapped *Peromyscus* sp. (from California), have been successfully infected using the St. Lawrence Island form.

TABLE 3.—*Measurements of Adult Specimens of* Echinococcus *from Alaskan Canine Hosts*

Host	Locality	Strobila length (in mm.)	Segment number	Hook size (in microns)		Egg size (in microns)	
				Large	Small	Range	Average
Dog	Point Barrow	2–3	2–3	.....	.....	35–40 × 28–32	37 × 29
Dog	Anchorage	3–4	3–4	.....	.....	32–37 × 30–32	35 × 30
Dog	Unalakleet	2–3	3	40	32–35	32–35 × 29–30	33 × 29
Dog	Point Barrow	Imm.	Imm.	35	24	.....	.....
Wolf	130 Miles N. E. Anchorage	2–2.2	2–3	.....	.....	34–40 × 29–34	36 × 31
Red fox	Nunivak Island	1–1.4	3	27	22	34–43 × 30–35	36 × 32
Red fox	Point Barrow	1	2–3	38	24	35–40 × 32–38	38 × 35
Dog	St. Lawrence Island	1.5–2.3	3–4	26–27	22	32–38 × 29–34	34 × 30
Arctic fox	St. Lawrence Island	1.2–1.9	3–4	29	25	30–34 × 29–30	31 × 29

Morphological differences between the adult stages of the two forms are not evident. In fact, the importance of adult morphology in the taxonomy of this genus of cestodes is not clear. The writer has at hand adult specimens representing at least six species of *Echinococcus*. These will be studied later in relation to the Alaskan material, and a separate report will be made. It is of interest to note the wide range of variation seen in the Alaskan specimens. This is summarized in Table 3. The following points are evident:

1. Strobila ranges in length from 1 to 4 mm., with segment number from 2 to 4 (rarely 5), when a gravid terminal segment is present.

2. Strobila size is possibly related to size of definitive host on mainland of Alaska. On St. Lawrence Island cestodes characterized by a very small strobila occur in both dogs and arctic foxes.

3. There is much variation, from one mainland locality to another, in size of rostellar hooks.

4. Form of gravid uterus is highly variable; it may range from branched to

sac-like. This character may be too variable to have value in species differentiation.

5. Egg-size of Alaskan material varies within definite limits (30-43 μ long by 28–38 μ wide); however, both range- and average-size variation differ in degree from one locality to another.

Clear-cut morphological differences are evident when the larval cestodes from St. Lawrence Island (from voles) are compared with those of the mainland form (from moose). Moose, so commonly parasitized by this helminth in southern Alaska, often show severe lung infections; the writer has observed as many as 18 cysts, up to 5 cm. in diameter, in the lungs of an old animal. Such cysts are typically spherical in shape, surrounded by a well-defined wall of host tissue-reaction. The germinal membrane is intimately associated with the surrounding connective-tissue capsule, but is readily freed when the cyst is incised. Many scolices occur in such a cyst, but they appear to arise directly from the germinal membrane; they are often attached to one another in groups by fragile strands of tissue, best seen in the living specimen. There has been no case observed where cyst formation tended toward any other structural arrangement. Secondary cysts within the large primary cyst have not been observed. In the moose, *Echinococcus* larvae occur regularly in the lungs, and the writer has not observed them in other locations in this host.

Larval cestodes observed in naturally-infected voles had characteristic multilocular structure, best described as an aggregation of small cysts, usually from 1 to 4 mm. in diameter, coalesced into an amorphous mass whose shape is largely determined by the character of the organ in which it occurs. The larval mass typically shows an evenly-granulated surface, but in some cases somewhat larger cysts may occur peripherally. Cyst size is apparently regulated by the process of reproductive growth.

While most experimentally infected voles *(Microtus californicus, M. pennsylvanicus,* and *Clethrionomys rutilus)* showed cyst growth identical to that seen in the naturally infected animals, one specimen of *M. pennsylvanicus,* killed 45 days after infection, showed an aggregation of larger, mostly single, cysts situated mainly on the parietal liver surface. Two other species of rodents, a muskrat *(Ondatra zibethica)* and a white-footed mouse *(Peromyscus* sp.*)* contained larvae of the same type. Since, in all cases, the larvae were normally developed (i.e., had produced large numbers of scolices), the significance of larval differences is not understood.

In every infected rodent so far examined, the invasion-center was the liver; beyond this, larvae were occasionally found in other organs, or attached along the mesenteries. Lung cysts in voles were never observed. In voles the liver is invaded in every case, and insofar as the writer has been able to determine, the liver infections precede all others. The secondary cysts must reach their site of localization after their derivation from the initial infection. This probably occurs through the rupture of earlier-established cysts, or possibly through metastasis. The latter method does not seem likely, but further study of the problem is necessary.

On the tenth day after experimental infection of voles, larval growth, seen as minute peripheral foci, was visible on the parietal liver surface. Voles killed and examined on the 20th day showed many isolated cysts of 1 to 3 mm. in diameter. These were well distributed throughout the organ. The study of these 20-day-old

infections showed clearly that reproduction occurred through the subdivision of the vesicle-like cysts. The primary cysts, each apparently arising from a single egg, showed internal septa which would result eventually in the production of many cysts from each. Scolex formation could not be observed on the 20th day after infection; however, after 40 days, scolices were numerous.

The rate of larval growth is surprisingly rapid in the case of vole infections, and this seems characteristic of the vole-infecting form of *Echinococcus*. A red-backed vole autopsied on the 40th day after infection disclosed a greatly enlarged liver, which caused extreme abdominal distension. This infected organ actually weighed more than the remainder of the animal's body. A similar situation was observed in a second animal, a specimen of *M. pennsylvanicus,* which finally succumbed to the combined effect of liver damage and pressure from the greatly-enlarged organ. Such animals are greatly hampered in their activity by the size of the infected liver, and no doubt in nature often become readily-captured prey for foxes and other animals feeding upon them.

Genus *Taenia* Linné, 1758

Two species of the genus *Taenia* whose larval stages are harbored by microtine rodents have been recorded from Alaska. These are *T. tenuicollis* Rudolphi, 1809, a common parasite of certain mustelids, particularly the ermine, *Mustela erminea arctica* (Merriam), and *T. crassiceps* Rudolphi, 1810, a parasite of the arctic fox, *Alopex lagopus innuitus* (Merriam), recorded here for the first time from North America.

Taenia tenuicollis Rudolphi, 1809

Abundant material of the larval form of *T. tenuicollis* has been collected from Alaskan voles and lemmings. These cysticerci were often observed in *Clethrionomys rutilus dawsoni, Lemmus trimucronatus alascensis,* and *Microtus miurus paneaki.* Comparisons of larval hook size and shape with these characters in the adult stage leaves no doubt as to specific identity. A limited range of variation in hook size was noted.

In earlier publications (Rausch and Tiner, 1949; Rausch, 1950), these cysticerci were tentatively assigned to *Cladotaenia.* This opportunity is taken to call attention to these erroneous reports.

It is necessary also to revise the earlier opinion that natural infections of *Cladotaenia* spp. are commonly observed. It is, on the contrary, more comparable to *Paruterina candelabraria* in regard to relative frequency of detected occurrence. Two possibilities are obvious: Either such larvae are overlooked in the rodent hosts, or the relatively high incidence of infection of hawks and owls by these cestodes is only the result of the consumption of an enormous number of rodents, among which infected animals are few.

Taenia crassiceps Rudolphi, 1810

Several hundred cysticerci of *Taenia crassiceps* were found free in the body cavity of a lemming, *Dicrostonyx groenlandicus richardsoni,* collected at Churchill, Manitoba. Another, but less heavy, infection was seen in a brown lemming, *Lemmus t. trimucronatus,* from the Melville Peninsula. *Taenia crassiceps* was the

most abundant cestode parasite of the arctic fox along the Arctic Coast of Alaska, but the larval stage has never been found in Alaskan rodents. Baer (1946) stated that its larvae are found "dans le tissu cellulaire sous-cutane de la region auxillaire ou inguinale" in rodents; this would lessen the probabilities of their being observed in the course of routine autopsy. The same author stated that the occurrence of these cysticerci in the host body cavity is rare.

The comparison of North American cestodes, both larval and adult stages, with European specimens, supplied by Dr. J. G. Baer, showed good agreement in morphological details. Another cestode parasite of European foxes, *T. polyacantha* Leuckart, 1856, closely resembles *T. crassiceps* in both adult and larval stages. The larvae may be differentiated, however, by the exogenus reproduction of *T. crassiceps*. This characteristic is well illustrated by Baer and Scheidegger (1946; p. 62). Although the asexual reproduction of the cysticerci was not as evident in the North American material as in that from Europe, it was nevertheless clearly-defined, and it is assumed that the large number of larvae recovered from the specimen of *Dicrostonyx* had resulted from this type of reproduction. A cysticercus of *T. crassiceps* from *Dicrostonyx* is shown in Fig. 10. A vial containing examples of these cysticerci has been deposited in the Helminthological Collection of the U. S. National Museum, accession No. 47804.

The record by Baer (1946) of the infection of a monkey by *T. crassiceps* is of considerable interest, since it gives further evidence that this cestode is not limited to rodent intermediate hosts. The possibility of human infection should not be disregarded, particularly since Eskimo-arctic fox relationships are often such that human exposures would be likely.

Since *T. crassiceps* has not been hitherto recorded from North America, a description of the adult cestode is included herewith. Hall (1920) reported *Taenia* sp. from the arctic fox on St. George Island (Pribilof group), but apparently no specific determination has been made of cestodes from this host species in North America.

Taenia crassiceps Rudolphi, 1810

(Figs. 11–13)

Diagnosis: Strobila length from 70 to 140 mm.; greatest width, slightly over 1 mm., attained in post-mature segments. Strobila margins serrate. Mature segments only slightly longer than wide; segment length increases toward end of strobila; gravid segments about four times as long as wide. Scolex relatively small, about 700 μ in diameter; oval suckers about 200 μ long. Rostellum armed with usually 30 hooks, arranged in two rows. Large hooks 172 to 178 μ long; small hooks 121 to 136 μ long. Genital pores irregularly alternate, situated in anterior half of segment margin. Genital papillae prominent. Cirrus sac from 160 to 215 μ long by 50 to 70 μ wide. Cirrus sac does not overlap ventral longitudinal excretory canal. Vas deferens highly coiled. Subspherical testes, about 220 in number, arranged in two lateral fields so as to have greatest number in anterior half of segment; testes distribution continuous across anterior part of segment. Narrow but unbroken row of testes extends from one field to the other just posterior to vitelline gland at posterior edge of segment. Testes about 50 μ in diameter in mature segments, do not overlap ventral longitudinal excretory canals; they closely surround bilobed ovary and vitelline gland. Vagina, opening posterior to cirrus sac, has uniform narrow diameter and takes direct course postero-mediad. Two reniform ovarian masses present; aporal one always larger. Each consists of relatively fine lobulations. Vitelline gland approximates ovarian masses in size. Ovary and vitelline gland situated completely in posterior segment-half. Uterus extends anteriorly into posterior part of preceding segment. Lateral uterine branches first appear as rounded outgrowths, which develop more rapidly at anterior end of main uterine stem. Lateral branches number about 20 on each side, often with terminal subdivisions. Eggs abundant, 25 to 32 μ long by 22 to 27 μ wide.

North American host: Arctic fox, *Alopex lagopus innuitus* (Merriam) and *A. lagopus* ssp.
North American distribution: Arctic Coast of Alaska and St. Lawrence Island.
Habitat: Small intestine of host.
A slide containing a whole-mount has been deposited in the Helminthological Collection of the U. S. National Museum, No. 47802.

The North American material does not correspond exactly in morphological detail to specimens from European foxes. The diagnosis given by Joyeux and Baer (1936) disagrees particularly in hook size and testes number. The differences, however, fall into the range of normal variation and are not considered significant.

II. TREMATODA

Genus *Quinqueserialis* Skvortsov, 1934

Four species of the genus *Quinqueserialis,* all parasitic in microtine rodents, are currently considered valid: *Q. quinqueserialis* (Barker and Laughlin, 1911); *Q. hassalli* (McIntosh and McIntosh, 1934); *Q. wolgaensis* Skvortsov, 1934; *Q. floridensis* Rausch, 1952. The genus was reviewed by Harwood (1939) and, more recently, by Ruiz (1946) in his revision of the family Pronocephalidae Looss, 1902.

Quinqueserialis quinqueserialis (Barker and Laughlin, 1911)

This trematode is a common parasite of Alaskan muskrats, but has been collected from voles *(Microtus pennsylvanicus drummondii* and *M. oeconomus macfarlani)* from only three widely separated localities (Arctic Village, Big Delta, and along the Skwentna River.) Adequate material was obtained to make possible comparisons with trematodes from both hosts collected in the United States. This opportunity is taken to make some remarks on relationships within the genus *Quinqueserialis.*

Few characters are of value in the differentiation of the species of this genus. Important among these are: number of glands in the ventral rows; form and distribution of the vitellaria; length of metraterm in relation to cirrus sac length; egg size. The North American species appear to be well characterized, although two of them show considerable morphological variation.

The Alaskan specimens of *Q. quinqueserialis* varied in size to a marked degree —the largest specimens occurred in voles, while relatively small ones were harbored by the muskrat. This was noted when specimens were collected from both muskrats and voles in the same locality. Size differences are often seen in material collected from muskrats in the United States; this is in part connected with the age of the trematodes.

Since the present work is concerned only with the mouse-like microtine rodents, the trematodes from muskrats were not studied in detail. The following observations were made on a good series of *Q. quinqueserialis* from *Microtus pennsylvanicus drummondii* from the Skwentna River near Anchorage, Alaska:

1. Considerable flexibility was noted in the number of glands in the ventral rows. The lateral rows had from 14 to 18; the paramesal rows, from 14 to 17; the median row, from 15 to 18.[2]

[2] Gland counts were made on trematodes stained entire with fast green and studied, unmounted, in terpineol.

2. The distribution of vitelline follicles ranged from that characteristic of *Q. quinqueserialis* to that seen in *Q. hassalli*—i.e., from a single row of follicles situated dorsal to the lateral gland row to more numerous follicles aggregated in a broader row, with at least a good proportion situated lateral to the lateral gland row.

3. The metraterm length ranged from 55 to 83 per cent of that of the cirrus sac.

4. Egg size ranged from 16 to 21 μ long by 8 to 11 μ wide; egg shape showed little variation.

It appears that the species *Q. quinqueserialis* tends toward intergradation with *Q. hassalli.* The number of glands in each ventral row has been thought to have particular diagnostic value, but even in Harwood's review of the genus, it was stated that this character is subject to considerable variation. False impressions have perhaps arisen through the failure to recognize variation of a local nature, such as is shown by other helminths for which a greater volume of material has been available for study. The writer is of the opinion at present that egg size and egg shape differ in the two species, *Q. hassalli* having a smaller and less elongate egg. Conclusions cannot be drawn at this time, but thorough study of these two species, preferably with experimental infections, should be made.

The status of *Q. wolgaensis* is somewhat questionable. Insofar as the writer is aware, it has not been recorded since it was described by Skvortsov (1934), who obtained it from *Arvicola terrestris* (L.) near Gorky, in the valley of the Volga River. From the description, it is evident that *Q. wolgaensis* is morphologically similar to both *Q. quinqueserialis* and *Q. hassalli.* Skvortsov was unaware of the existence of *Q. hassalli* when he prepared the description of *Q. wolgaensis,* since he stated (page 321) that ". . . only one American species, *Notocotylus quinqueserialis* Barker and Laughlin, 1914, has five rows of them" [i.e., ventral glands]. Skvortsov's figure 1 (page 319) and his description do not support Harwood's statement (1939; p. 430) that *Q. wolgaensis* may be separated from the two closely-related species on the basis of vitelline follicle distribution. Skvortsov stated (page 321) that his species differed from *Q. quinqueserialis* ". . . in shape of the body, in the number of papillae in the longitudinal series, in the size of the bursa of cirrus and in the size of the vagina."

In the original description, *Q. wolgaensis* has 14–15 glands in the middle row and 15 in paramesal and lateral rows. Although Skvortsov had 81 specimens of this trematode, it is possible that he failed to make gland counts on a large enough series to determine the ranges. According to the original figure, the metraterm is about half the length of the cirrus sac; this ratio is subject also to considerable variation. The eggs of *Q. wolgaensis,* 18 to 22 μ long by 9 to 12 μ wide, are almost identical in size with those of *Q. quinqueserialis.*

One will not be able, without material for comparative study, to determine the status of *Q. wolgaensis;* the possibility should be kept in mind, however, that *Q. wolgaensis* is conspecific with *Q. quinqueserialis. Q. quinqueserialis* may occur naturally in Eurasia, but in any event the muskrat, along with its parasites, has been widely introduced there from North America. Warwick (1936), for example, has recorded *Q. quinqueserialis* from the muskrat in Great Britain.

Genus *Plagiorchis* Lühe, 1899

Plagiorchis sp.

Two specimens of *Plagiorchis* sp. were taken from the small intestine of a red-backed vole, *Clethrionomys rutilus dawsoni,* trapped near Anchorage, Alaska. In North America, only *P. proximus* Barker, 1915, from the muskrat, has been recorded from microtine rodents. Two species of *Plagiorchis* have been reported from Eurasian voles; these are *P. arvicolae* Shul'ts and Skvortsov, 1931, from *Arvicola amphibius* L., and *P. microti* Soltys, 1947, from *Microtus arvalis* Pallas. *Plagiorchis* sp. was also reported by Warwick (1936) from *A. amphibius* in the British isles.

The Alaskan vole specimens in some respects resemble *P. proximus* Barker, 1915, which occurs commonly in Alaska as a muskrat parasite. A brief description of the trematodes from the vole is included herewith (Fig. 14):

Length 1.5 mm. Subterminal oral sucker equal in size to acetabulum; latter in first body third. Anterior edge of ovary at posterior margin of acetabulum. Testes and ovary nearly equal in size. Vitellaria extend from posterior end of body to beyond posterior margin of oral sucker; laterally they overlap testes' margins and extend dorsally nearly across body width at anterior limit of their distribution. Cirrus sac, about 320 μ long, extends from genital pore posteriad beyond anterior margin of ovary. Uterus typical. Eggs, somewhat distorted, measure about 34 × 16 μ.

A whole mount has been deposited in the Helminthological Collection of the U. S. National Museum, Slide No. 47812.

Genus *Brachylaima* Dujardin, 1843

Brachylaima rauschi McIntosh, 1951

This trematode was observed only as a very local parasite of microtine rodents. Few specimens were obtained, and nothing can be added here to the original description. An erroneous type locality was designated; this should be Tulugak Lake, Anaktuvuk Pass, in the Brooks Range. In addition to the type host, *Dicrostonyx groenlandicus rubicatus,* this trematode was also collected from *Microtus miurus paneaki.*

Trematodes of the family BRACHYLAIMIDAE apparently do not often parasitize microtine rodents. The writer has obtained *Brachylaima* sp. from *Microtus pennsylvanicus terraenovae* Bangs, Penguin Island, Newfoundland; these specimens, six in number, have been given to Mr. Allen McIntosh, Zoological Division, Burean of Animal Industry, for study. *Entosiphonus thompsoni* Sinitsin, 1931, was reported by Rausch and Tiner (1949) from *M. p. pennsylvanicus,* from southern Wisconsin.

III. NEMATODA

Genus *Heligmosomum* Railliet and Henry, 1909

Heligmosomum costellatum (Dujardin, 1845)

Heligmosomum costellatum was first reported from North American rodents by Kuns and Rausch (1950), who collected it from voles trapped in subalpine habitats in the Rocky Mountains of Wyoming. The present work has shown it to be a widely distributed parasite of various species of voles in Alaska. Since a detailed description of this nematode was given in the earlier paper (1950), nothing further is included here.

Heligmosomum hudsonius Cameron, 1937

This species was described from "*Dicrostonyx hudsonius*" from northern Quebec and Baffin Island by Cameron (1937). The original material had been preserved when it came into Dr. Cameron's hands, so no observations were made on the living organism. Nothing can be added here to the original description, except for remarks on the living worms.

The writer has collected *H. hudsonius* from a single locality in Alaska. A heavy infection was observed in *Dicrostonyx groenlandicus rubricatus* collected at Tulugak Lake in the Brooks Range. The nematodes were tightly spiralled around the greatly elongate cecal villi of the lemming, and did not release their attachment when placed *in situ* into cold water. The nematodes were blood-red in color when alive.

Genus *Nematospiroides* Baylis, 1926

Nematodes of the genus *Nematospiroides* are rather common parasites of voles over most of North America. *N. longispiculatus* Dikmans, 1940, appears to be the most widely distributed. However, it would be desirable to determine the limits of variation in the species of this genus, in order to be able to characterize them adequately. The writer is of the opinion that morphological variation in this group is much greater than is generally recognized at present.

Mr. Merle Kuns, Department of Biology, Purdue University, has studied the Alaskan *Nematospiroides* material in connection with his review of the family Heligmosomidae, and the writer is indebted to him for the identifications included here. His work has been delayed in its completion by military service; for that reason specific names cannot be included in some cases.

Nematospiroides longispiculatus Dikmans, 1940

The only record of *N. longispiculatus* was obtained at Juneau, where it was found in a common vole, *Microtus longicaudus littoralis*. It apparently is replaced farther north by another species.

Nematospiroides spp.

A single species of *Nematospiroides* was found over most of Alaska as a parasite of *Microtus* spp. The description of this nematode has been completed by Mr. Kuns, who will publish it separately.

The writer collected a few specimens of *Nematospiroides* from *Lemmus trimucronatus alacensis*, at Point Barrow. These specimens may represent a distinct species. Mr. Kuns made the following comment (personal communication) on these: ". . . *Nematospiroides* sp. [from the lemming] is similar to [that from Alaskan voles] except for generally larger size in nearly all respects. More material is needed on the latter so that an effort can be made to determine if this is only a host difference rather than a specific one." These specimens will be considered in detail in the published report of Mr. Kuns.

The writer has not collected *Nematospiroides* from voles in the localities where it occurs in lemmings. If this is eventually accomplished, it should permit better evaluation of the size differences.

Genus *Syphacia* Seurat, 1916

Syphacia obvelata (Rudolphi, 1802)

Over most of Alaska, *Syphacia obvelata* is an abundant rodent nematode parasite. It is found wherever voles occur, and its absence along the central Arctic Coast region can be correlated with vole distribution. No discussion of the morphological details of this species is justified, since specimens collected were typical.

Syphacia arctica Tiner and Rausch, 1950

Of the lemmings *(Dicrostonyx)* examined at Point Barrow, a good proportion was found to be infected by *Syphacia arctica.* In addition, one infected collared lemming was collected at Tulugak Lake, in the Brooks Range. Large numbers of brown lemmings *(Lemmus)* were also examined along the Arctic Coast, but this parasite was not found in them; it is considered, consequently, to be specifically adapted to *Dicrostonyx.* This is one of the few instances where, in connection with this study, evidence for rigid specificity of an helminth parasite for a given host has been obtained. Nothing can be added to the original description in way of morphological details.

Genus *Rictularia* Frölich, 1802

Nematodes of the genus *Rictularia* are important parasites of several mammalian groups (see Dollfus, 1944–45). In North America, sciurids frequently harbor nematodes of this genus, but microtine rodents are rarely parasitized by them. The report by Rankin (1945) of a single specimen of *R. coloradensis* Hall, 1916, in *Microtus longicaudus,* in Washington, apparently constitutes the only record. *Rictularia* occurs locally in Alaskan voles, but has not been found in sciurids *(Citellus parryii)* in the same localities.

Rictularia microti McPherson and Tiner, 1952

Massive *Rictularia* infections were observed in St. Lawrence Island voles, where as many as 119 nematodes were taken from a single animal. As is characteristic of the group, male individuals were relatively few; in the two heaviest infections noted, with 112 and 119 worms, eight and nine males, respectively, were present. The material from St. Lawrence Island formed the basis for the description of *R. microti* by McPherson and Tiner (1952).

Rictularia sp. was recorded several times from *Microtus miurus paneaki* and once from *Clethrionomys rutilus dawsoni* at Tulugak Lake, Brooks Range.

It has not been determined whether the Brooks Range specimens are conspecific with *R. microti.* In reference to the former *(M. miurus paneaki),* Tiner (personal communication) stated: "In general, specimens from *M. miurus paneaki* were in agreement with the Island males and females from *M. oeconomus innuitus.* However, the buccal cavity was circular in cross section in both sexes from the mainland, whereas the stoma of the male from the Island had a circular cross section and that of the female was roughly oval. The long axis of this oval extended dorsoventrally. There were about 20 denticles in the members of each sex from the mainland. One male from the mainland had four pre-cloacal fans and spicules about 160 μ long. Two other males agreed with those from the Island in that they lacked pre-cloacal fans." He also is of the opinion that ". . . it would be highly in-

advisable to speciate *Rictularia* on the basis of the male specimens until much larger series have been studied for each species."

Since the Brooks Range specimens were obtained from voles trapped in a very restricted sedge-bog habitat, adequate material may not be obtainable. The fact that both forms occur in voles suggests a close relationship. Tiner has pointed out the possibility that the periodic fluctuations in vole populations would greatly influence the nematode populations also; the chance survival of individuals with certain characters might result in a relatively homozygous and uniform species on St. Lawrence Island, since it is completely isolated from the Alaskan mainland.

Genus *Mastophorus* Diesing, 1853
Mastophorus muris (Gmelin, 1790)

Mastophorus muris was a locally common parasite of red-backed voles in Alaska. It was restricted to spruce forest habitats. In the writer's experience, *M. muris* is not a general parasite of North American voles; in Michigan, where a few infected voles were collected, it was found in but one locality (Rausch and Tiner, 1949).

In reference to *Clethrionomys rutilus dawsoni* on the Seward Peninsula of Alaska, Quay (1951) reported that ". . . some of the vole stomachs contained many large nematodes." It seems probable that these were *M. muris*. This nematode was recorded from *C. rutilus* in Norwegian Lapland by Baylis (1931).

Protospirura glareoli Sołtys, 1947 [= *Mastophorus glareoli* (Sołtys, 1947)] was described from *Clethrionomys glareolus* Schreber, in Poland. It is differentiated from *M. muris* on the basis of position of vaginal opening, spicule length, and labial characters.

Genus *Capillaria* Zeder, 1800
Capillaria sp.

One specimen of *Capillaria* sp. was collected from a red-backed vole, *Clethrionomys rutilus dawsoni,* trapped at Kotzebue, Alaska. Specific determination was not possible. From Bering Island, in the Komandorskii group, a single specimen, also unidentified, was obtained by the writer from one of three voles of the same species.

Only *C. muris-sylvatici* (Diesing, 1851) has been recorded from Nearctic voles. It occurred as a locally-common parasite in southern Wisconsin (Rausch and Tiner, 1949). Intensive studies in other regions will no doubt disclose the presence of this species, as well as others.

Unidentified nematode

A minute nematode was obtained from the eyes of brown lemmings at Point Barrow. These were first collected by Mr. Daniel Q. Thompson, of the University of Missouri, who observed them during the course of his studies on lemming ecology. Present material does not permit identification; however, the writer expects to investigate this parasite further at Point Barrow in the spring of 1952. Information presently available indicates a direct life cycle.

SUMMARY

An investigation of the helminth parasites of microtine rodents of Alaska and northern Canada has been carried on for three years. A total of 2078 rodents of 26

species and subspecies was examined. Twenty-eight species of helminths were considered, of which *Paranoplocephala lemmi* n. sp., and *Andrya arctica* n. sp., (type locality for both Point Barrow, Alaska), were described. *Taenia crassiceps* Rudolphi, 1810, was recorded for the first time from North America. Taxonomic discussions are included for each species considered.

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Explanation of Plates

Figures drawn by Miss Reggie V. Sacressen, technical assistant, Animal-borne Disease Branch, Arctic Health Research Center. All figures from projected whole-mounts.

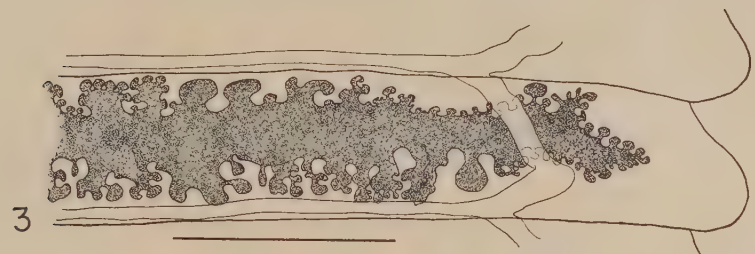
Plate I

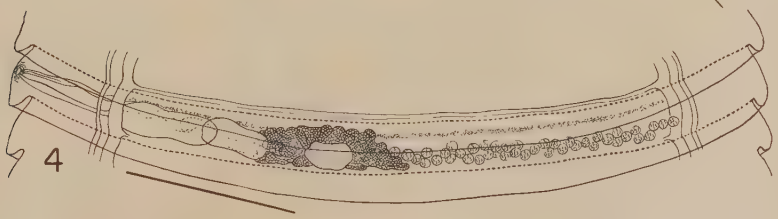
Fig. 2. *Paranoplocephala omphalodes,* mature segment. Scale has value of 500 μ.
Fig. 3. *P. omphalodes,* pregravid segment, showing characteristic uterus development. Scale has value of 500 μ.
Fig. 4. *P. lemmi* n. sp., mature segment. Scale has value of 250 μ.
Fig. 5. *P. lemmi* n. sp., scolex. Scale has value of 500 μ.
Fig. 6. *P. lemmi* n. sp., entire strobila. Scale has value of 5 mm.
Fig. 7. *P. lemmi* n. sp., details of cirrus sac and vagina. Scale has value of 500 μ.

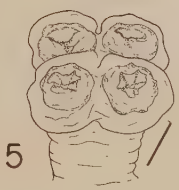
Plate II

Fig. 8. *Andrya arctica* n. sp., mature segment. Scale has value of 500 μ.
Fig. 9. *A. arctica* n. sp., scolex. Scale has value of 200 μ.
Fig. 10. *Taenia crassiceps,* cysticercus. Scale has value of 500 μ.
Fig. 11. *T. crassiceps,* mature segment. Scale has value of 250 μ.
Fig. 12. *T. crassiceps,* rostellar hooks. Scale has value of 50 μ.
Fig. 13. *T. crassiceps,* gravid segment. Scale has value of 1 mm.
Fig. 14. *Plagiorchis* sp. Scale has value of 500 μ.

PLATE I







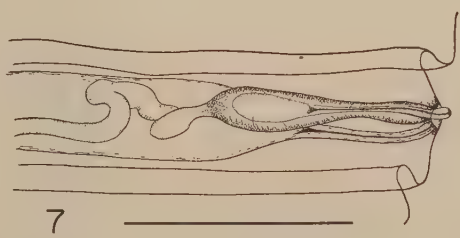
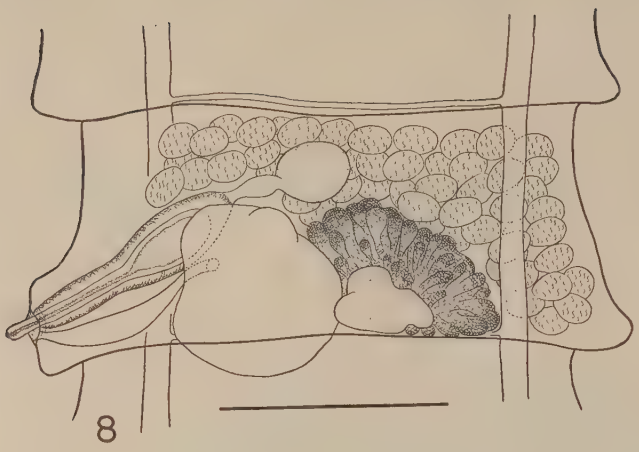


PLATE II



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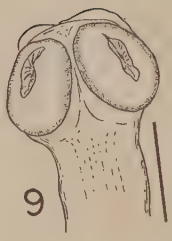

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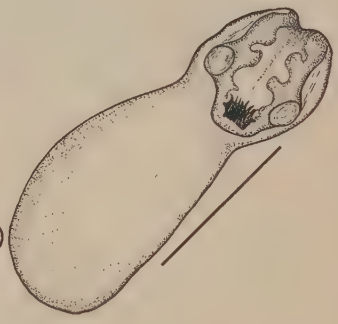

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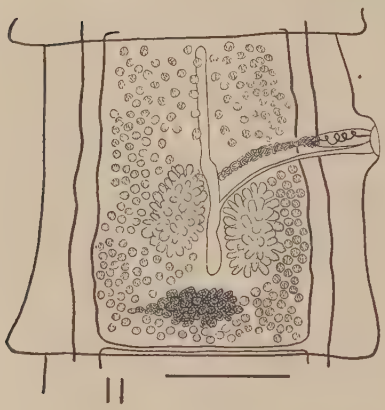

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OBSERVATIONS ON THE EPIDEMIOLOGY OF ASCARIASIS IN A REGION OF HIGH HOOKWORM ENDEMICITY[1]

PAUL C. BEAVER[2]

In regions of the United States where ascariasis is most prevalent, hookworm (*Necator americanus*) infections are uncommon or absent. Conversely, in regions where hookworm infection is hyperendemic, as in the lower coastal plain of the southeastern United States, the incidence of ascariasis is generally low. Clay and other types of dense soils which predominate in ascaris areas are known to be unsuitable for the development of infective hookworm larvae and thus constitute one of the principal limiting factors (Augustine and Smillie, 1926; Rickard and Kerr, 1926). The factors responsible for the low incidence of ascariasis in regions where conditions are favorable for hookworm infection have not been well established. The principal causes which have been suggested by previous studies are the sandy character of the soil and peculiar defecation habits of the people (Otto and Cort, 1934). The present study is a further inquiry into these factors.

MATERIALS AND METHODS

The investigation was carried out during the summer of 1950 in southeastern Georgia, mostly in the vicinity of Waycross. The selected region was regarded as typical of the lower coastal plain and the period was climatologically typical of the season (Figs. 1, 2,). The study concerned (1) ascaris-infected families, (2) the infectivity of the soil in their dooryards, (3) the rate and level of ascaris infection in pigs, and (4) the rate of development, survival and distribution of ascaris eggs in the soil.

Data on the family were obtained by visits to the homes of ascaris-positive individuals selected from groups in which recent surveys had detected 82 ascaris infections, 50 of which occurred as double infections along with hookworm, among 5,028 school children, 52 per cent of whom were carrying hookworm infections. Fecal examinations on 994 other individuals of all age groups had revealed hookworm infections in 58 per cent and ascaris in 43 individuals, 36 of whom also had hookworm infections. For dooryard studies 20 to 25 gm. samples of soil were taken by scraping small portions from numerous locations and eggs were recovered from the samples as follows: To each part of soil 2 parts of 30 per cent commercial bleaching fluid (6 per cent sodium hypochlorite) were added and allowed to stand for 30 minutes with frequent stirring. The mixture was then diluted, filtered through coarse gauze, washed by centrifugation and separated by brine flotation. The top film of the flota-

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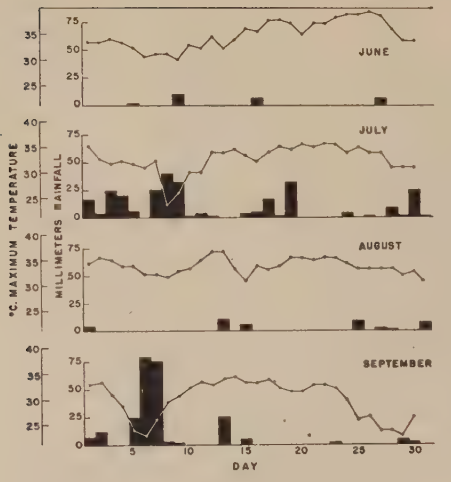

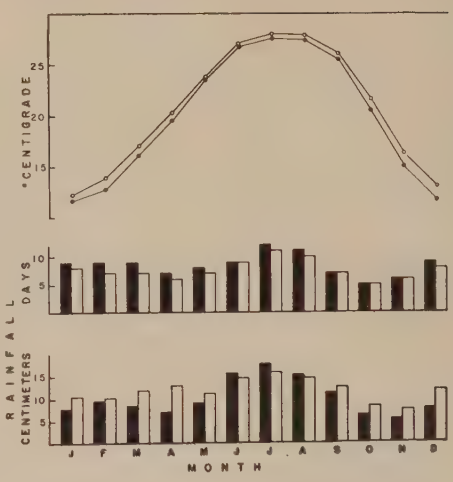

FIG. 1 (Left). Maximum daily temperatures and rainfall for the period June 1 to September 30, 1950, at Waycross, Georgia, based on United States Weather Bureau reports.

FIG. 2 (Right). Normal monthly mean temperature, rainfall, and number of days with rainfall for southern Georgia (closed circles and bars) and southern Louisiana (open figures). Temperature and total rainfall data are for Waycross, Georgia, and New Orleans, Louisiana; number of days of rain are for the respective regions, based on United States Weather Bureau reports.

tion was then collected, diluted and centrifuged, and the sediment was examined microscopically. Stool examinations were made by brine flotation and counts of the ascaris and hookworm eggs were made in standard smears (Beaver, 1950).

RESULTS

1. *The Family.* Visits were made to the homes of 50 families in which one or more members were infected with ascaris. Hookworm infection among these families was roughly equal to that of the rest of the population, being present in 4 of 17 negro families and 24 of 33 white families. Individually, hookworm infections were found in 14 per cent of 90 negroes and 51 per cent of 193 white members. Ten per cent of the infected white group and none of the negroes had egg counts above 20/mg. of feces; moderate infections (5–20 eggs/mg.) were found in 35 per cent and 8 per cent of the infected members of the two groups respectively.

The ascaris-infected families were mostly large (average, 6.8 members) and in all but 7 of them there were children under 5 years of age. The ascaris families were about equally distributed in town, village and farm environments but frequently had moved from one to the other. Almost half of them had moved one or more times within the past year. However, none had immigrated from distant locations outside the hookworm endemic region. In their present locations 11 had flush toilets, 12 had pit privies, 17 had open-back surface privies and 10 had no provisions for the disposal of feces. Relationships between sanitary facilities and the degree of ascaris infection in the family were not evident.

Due largely to coprophagous animals, especially dung beetles, stools were infrequently found near the houses and were invariably fresh when seen. However,

over half of the mothers stated that the younger children were permitted to defecate in the dooryard. Although all were familiar with the mode of hookworm infection, only 8 of the 50 mothers had similar accurate knowledge of ascaris. Thirty-nine of the group stated that the family had been infected previously, and in 28 of the families spontaneous passage of worms had been observed.

Stool examinations were made between June 17 and July 19. Results for ascaris infections, reported by age groups 1–4, 5–9, 10–19 and adults respectively, were: number examined—75, 82, 55 and 80; per cent positive—75, 71, 42 and 15; percentage of positives with egg-counts above 100/mg. of feces (uncorrected for stool size)—34, 21, 13 and 0; percentage of positives below 25/mg. of feces—21, 55, 52 and 75; average egg-count of positives—84, 50, 35 and 19. Differences between the negro and white groups were not significant.

Clay topsoil is uncommon in the area studied. However, 11 families were either living where the dooryard soil contained appreciable amounts of clay or had moved from such a location within the past year. Egg counts gave no clear indication of differences between ascaris infections among this group and the families living on sandier soils. While clay and loam soils are characteristically dense and pack readily, it was noted also that in all but 7 of the sandy dooryards, due largely to the accumulation of household refuse, the soil was packed at the entranceways of the houses and was sufficiently dense to hold considerable moisture when other areas were dry.

2. *Infectivity of Dooryard Soil.* Soil samples were collected from the dooryards of 7 ascaris families at the end of the dry season (June 22–28) and from 10 similar dooryards during the fourth week of the rainy season (July 22–28). The samples were taken from various locations but positive ones came mostly from moist shaded areas, especially at the stoops, or entranceways, of the houses. Dry season samples from the front and back stoops of 4 of the houses contained 2–70 viable eggs and in 4 of the samples, from 3 different yards, 1–12 of the eggs contained motile larvae. During the rainy period, up to 37 (average, 8) eggs with motile larvae were found in the samples taken at the stoops (front, back, or both) of all but one of the houses and viable eggs in cleavage stages were found at each house, although 3 of the 20 individual 25 gm. samples were negative. In general, the poorest yields were obtained from the areas that were least shaded.

Correlation between the yields from soil and the sanitary provisions or the number, ages, egg-output and length of residence of infected members of the family was not evident. There was, on the other hand, a marked difference between dry-season and wet-season samples. However, at locations where refuse from the house was sufficient to maintain dampness, ascaris eggs remained viable even in dry periods. It is of interest that during the dry season, samples from near the surface of this refuse-contaminated zone contained active free-living nematodes, rotifers, tardigrades and annelids. Viable eggs of *Enterobius vermicularis* and *Trichuris trichiura* were found in 2 samples each, and *Toxocara canis* eggs containing active larvae were found once (in each of 3 samples) during the dry season and in 5 samples taken from 3 different dooryards during the rainy season.

3. *The Pig Ascaris.* Between July 1 and July 28 the small intestines of 212 pigs from nearby farms were examined for ascaris at a small slaughterhouse in Waycross. Since the intestine was emptied and washed for use as sausage casing, it was

possible to recover the worms individually from all of the animals slaughtered during the period and to obtain essentially all worms that were 20 mm. or more in length. The pigs weighed 145 to 250 pounds and were estimated to be 6 to 8 months of age.

Seventy-one, or 33 per cent, of the animals harbored 1 to 22 worms. Females alone were found in 32, males alone in 11, and immature worms alone in 6 of them. The infections averaged 3.5 worms and only 2 animals harbored more than 5. There were 5 pigs from which only small worms, 20 to 50 mm. in length, were recovered and 4 others in which similar small worms were found among mature ones. The small worms were not found until the fourth week of the rainy season.

4. *Development, Survival and Distribution of Eggs in Soil.* Pooled fresh human stools containing ascaris eggs were stirred into a watery suspension which, when divided into 4 portions, resembled large (200 cc.) diarrheic stools bearing approximately five million eggs each. On July 1, the material was poured onto the soil in single masses located as follows: (1) under a broad-leafed tree which provided almost complete shade, where the soil was approximately 90 per cent fine sand with a small amount of silt and dark organic material; (2) under a tall tree which, along with tall broad-leafed weeds, provided almost complete shade, where the soil contained an appreciable amount of clay in addition to silt and sandy elements (80 to 85 per cent sand); (3) 45 cm. from the east face of a solid fence where the sandy soil was coarse and clean (95 per cent sand) and the vegetation provided negligible shade; and (4) under the west eaves of a building where the soil was essentially the same as in the first area and without vegetation. The experimental areas thus represented a variety of conditions characteristic of the region and will be referred to as shaded sand, shaded clay, coarse sand with afternoon shade and fine sand with morning shade. It will be noted, however, that the "shaded clay" was actually a sandy soil bearing 15 to 20 per cent silt and clay. The egg-bearing feces were made watery in order to avoid interference by dung beetles and to insure that observations would not be complicated by irregular liberation of eggs from the feces. Excepting some fiber and seeds, the fecal elements were entirely dissipated by the first rain. As a control on the rate of development, eggs were concentrated from feces by brine flotation, washed and incubated at room temperatures in a closed petri dish containing 1 per cent formalin about 3 mm. deep. Percentage computations were based on the examination of 100 eggs or more.

Development was uniformly rapid in the control. At 10 days, 80 per cent of the eggs contained motile larvae and the others were in advanced tadpole stages. At the same time, eggs from the soil plots were not beyond the late gastrula. After 20 days, all fertile eggs in the control contained motile larvae, in the clay plot 91 per cent of viable eggs contained motile larvae, and in shaded sand about half had reached this stage. In the plots exposed to the sun, either morning or afternoon, a large proportion of the eggs were dead (97 per cent from the coarse sand) but all survivors contained motile larvae. By contrast, in shaded sand and clay plots respectively, 90 and 98 per cent of the recovered eggs were viable. Survival in terms of percentages could not be followed beyond 20 days because even in this period it became difficult to recover and recognize the remains of dead eggs. After a 30-day period of frequent rains, eggs containing viable larvae were still numerous in surface scrapings from the shaded sand. Ninety days after inoculation, an occasional egg with viable larva could be found in the most protected sandy areas. In shaded

clay, on the other hand, eggs with motile larvae were moderately numerous in spite of two intervening periods of drought and one period of extremely heavy rainfall (Fig. 1). However, even here there was a reduction of approximately 97 per cent below the density at 20 days (Table 1).

On areas too level to permit appreciable transport by water currents, a number of viable eggs were found 50 to 75 cm. from their original locations and one was found in a sample taken at a distance of 125 cm. 20 days after inoculation. A large proportion of the eggs became buried in the clay soil while those placed on sandy soils tended to remain at the surface or accumulate on splashed surfaces above the ground. At 20 days after inoculation, however, at least some eggs were found 4 to 10 mm. below the surface in all plots. After 90 days, eggs were found in sub-surface samples taken only from clay. At the shaded sand plot, on the vertical surface of smooth boards which were 30 cm. from the inoculated site and were respectively

TABLE 1.—*Viable eggs in 10 gm. samples taken from various locations with reference to the original site of inoculation. Samples from above the ground level were 3 gm. or less. For rainfall data see Figure 1. Inoculations were made on July 1, 1950*

Plot	Days after inoculation	cm. from site in upper 2–3 mm.		4–10 mm. below surface	6–30 cm. above surface
		0	50–75		
Shaded sand	20	540	13	..	1,200
	30	309	..	36	495
	90	3	0	0	0
Shaded clay	20	4,180	32	19,360	21
	90	133	42	876	..
P.M. shaded coarse sand	20	66	8	150	..
	90	0	0	0	..
A.M. shaded fine sand	20	325	270	..	..
	90	4	13	..	..

6 cm. and 30 cm. above the ground, ascaris eggs along with minute soil elements formed a persistent layer of splashed material. About 3 gm. of this material scraped from the lower board at 30 days contained eggs more abundantly than 10 gm. samples from the soil surface; earlier, at 20 days, about 1 gm. brushed from the upper board contained 28 viable eggs and numerous dead ones. Similarly, 21 viable eggs were recovered from a small quantity of material rinsed from 4 leaves (about 100 sq. cm.) approximately 25 cm. above ground over the shaded clay plot (Table 1).

DISCUSSION

Studies by Cort and his associates (Brown, 1927a; Cort, 1931; Cort and Otto, 1933; Otto and Cort, 1934) and by Headlee (1936) have shown that ascariasis is essentially a household infection. It is an infection that is spontaneously lost and repeatedly reacquired from infective eggs in dooryard soil, and the infectivity of the soil is maintained for the most part by promiscuous defecation by the younger members of the family who are themselves almost always the most heavily infected. For this reason, and because eggs may remain infective for relatively long periods in the soil, facilities for waste disposal generally are not of prime importance in the maintenance of infections within the family. In considering this feature of the ascaris-infected family, perhaps the role of the diaper-age child should be given additional emphasis. It was noted in the present study that not only was there a general

willingness to permit promiscuous defecation by the younger children, but adults also contributed significantly to the pollution of dooryard soil by habitually discarding egg-laden laundry wastes near the house.

Nothing remarkable was observed regarding the size, origin and general character of the families. Ascariasis, though relatively uncommon, is indeed endemic, since apparently all of the infections were acquired within the region. It is noteworthy that the "ascaris family" is oftentimes also a typical "hookworm family." Unpublished results of soil sampling from the dooryards of hookworm-infected families indicate that, although there undoubtedly is important soil pollution at more distant sites, dooryard pollution is of common occurrence among this group. It is thus doubtful that the scarcity of ascaris in the hookworm region is due to peculiar defecation habits of the people. At least this would be true as regards the younger children.

Because recent experiments by Takata (1951) have shown that the pig ascaris is capable of reaching maturity in man, it is unfortunate that data were not obtained on the degree or recentness of contact between members of the study group and pigs. Since less than half of the families were rural and not all of them were engaged in farming, it can be assumed that relatively few could have had significant direct contact with ascaris infected animals. The infection rate of 33 per cent in pigs with an average of 3.5 worms per infection and a maximum burden of 22 is somewhat lower than has been found in other regions (Ransom and Foster, 1920; Allen and Jones, 1949; Caldwell and Caldwell, 1926; Spindler, 1934). Of special interest are the findings of Andrews and Connelly (1945) who recovered an average of 7 worms from 68 per cent of 129 pigs that were raised under moderately sanitary conditions in the upper coastal plain of Georgia, where the predominant topsoil is sandy loam and hookworm infections, though common, are much less frequent and lighter than in the lower counties. As far as can be judged from the limited available data, the rate of ascaris infection in pigs, as in the human population, is lower in the sandy coastal plain than in climatically similar regions where heavier soils predominate. However, more information is needed, especially with reference to seasonal variation. The low sexually unbalanced worm population carried over from the dry season and the appearance of young worms 3 to 4 weeks after the beginning of the rainy season suggest the possibility of seasonal fluctuation of the ascaris infections in the pig, as well as in the human population as has been observed in Panama (Cort *et al.*, 1929).

The refuse-laden, draught-resistant zone around the stoops is undoubtedly a factor in maintaining human ascariasis in the sandy region. However, it generally constitutes only a small portion of the dooryard and would persist for only a short period at disinhabited houses. It serves, therefore, as a source of infection and reinfection within the family but would be of minor importance in the transfer of infection from one family to another. Possibly an explanation is thus offered for the fact that despite the scarcity of ascariasis in the study area, the infections, in terms either of frequency of high egg-counts or of average egg-counts for the different age groups, were not notably light as compared with those of highly endemic areas in other parts of the United States (Otto and Cort, 1934).

A fact which appears to have been well established by earlier observations (Cald-

well and Caldwell, 1926, 1928; Brown, 1927b; Otto, 1929; Spindler, 1929) and confirmed by the present study is that, due to their susceptibility to heat and drying, ascaris eggs survive for relatively shorter periods in sandy than in heavier types of soils. Recent observations indicate that even in the more drought resistant soils they generally survive shorter periods than was formerly thought probable (Rudolfs *et al.*, 1951; Gartner and Müting, 1951). Soil erosion studies by Laws (1940) and Ellison (1947, 1950), summarized by Stoltenberg (1950), are helpful in the further interpretation of these observations. They observed that the force of falling raindrops loosens and breaks up aggregates of the soil particles, suspends and rearranges them by sedimentation and by splash transport. The intensity of the several effects is, of course, determined by the number, size and velocity of the drops. Thus, the total precipitation is of less importance in this regard than the character and frequency of the rainy periods. Soil particle disturbance by rain being greater in the more open soils, the dispersal and eventual destruction of ascaris eggs likewise would be more extensive in sandy soil. Ascaris eggs are among the lighter elements of sandy soils. Clay and loam soils, on the other hand, contain a high proportion of fine colloidal elements lighter than the eggs. In suspensions of sandy soil, therefore, the eggs tend to sediment to the topmost layer while from suspensions of denser soils they sediment to subsurface strata beneath a buffering layer of the lighter elements. This action probably is of further significance in that eggs concentrated in a shallow subsurface stratum of adhesive colloidal material would be ideally situated for transport into the houses or from dooryard to dooryard *via* muddy feet.

In regard to adaptation to sandy soil, perhaps the most fundamental difference between ascaris and the hookworm is that the infective stage of the hookworm is reached after a relatively short period of incubation and its early developmental stages become motile soon after the eggs reach the soil, whereas the infective stage of ascaris requires much longer incubation and is non-motile in all developmental stages. For this reason, desiccation as a result of rapid drying at the surface of sandy soils following rains is a hazard of relatively minor importance to the negatively phototropic pre-infective hookworm larvae and one of great importance to ascaris. Furthermore, if ascaris eggs are deeply buried in the soil, as they frequently are by dung beetles (Miller, 1952), they are more or less permanently out of reach of prospective hosts. The same event possibly would increase the chances of new infection by the hookworm. When splashed onto elevated surfaces, the infective stages would be benefited in both species by temporary enhancement of transfer to new hosts but there would also be increased exposure to natural hazards which possibly would be less unfavorable to the motile hookworm larvae.

It remains to be determined to what extent ascaris and hookworm infections are acquired during the colder months in the coastal plain of Georgia and adjacent areas. It is evident, however, that in the warm period of the year conditions favorable for their dissemination prevail only during a relatively short midsummer rainy season. Because the average life span of *Ascaris lumbricoides* is relatively short, whereas *Necator americanus* infections normally persist for several years, brief warm rainy periods interrupted by long dry or cold periods exert a differential effect favorable to the latter species. However, climatic conditions *per se* in southeastern Georgia apparently are not unfavorable for ascaris. The only highly endemic ascariasis region of the United States in which there is greater normal sum-

mer rainfall is Tampa, Florida. In southern Louisiana where the incidence of ascariasis is high and hookworm infection is rare (Otto, 1932; Headlee, 1936), the rainfall and temperature conditions are essentially the same as those of southeastern Georgia (Fig. 2). The soils, however, are distinctly different in the two regions, being dense colloidal moisture-retaining varieties in the former and light open sandy types in the latter. Apparently, one of the most fundamental of the variables which affect the distribution of both ascaris and hookworm in the unsanitated areas of the southern United States is the character of the topsoil. Clay and sandy soils may be regarded as culture media, serving respectively as favorable and unfavorable environments for the developmental stages of ascaris, and in reverse manner for the hookworm.

SUMMARY AND CONCLUSIONS

1. To determine some of the factors responsible for the low incidence of ascariasis in a region of high hookworm endemicity, observations were made on 50 ascaris-infected families, on the infectivity of dooryard soil, the rate and level of ascaris infection in pigs, and the rate of development, survival and distribution of eggs in the sandy soils of the lower coastal plain of southeastern Georgia.

2. The ascaris-infected families were found to be typical also of the hookworm-infected families of the same region.

3. Viable ascaris eggs, usually some with motile larvae, were found in the dooryards of all infected families, but during the dry season they were relatively scarce and were found only in a narrow zone around the entranceways where dampness was maintained by household refuse.

4. Ascaris infections in pigs were lighter and less frequent in the study area than in a neighboring area where denser soils predominate.

5. In 4 experimentally inoculated plots, ascaris eggs developed more rapidly and survived better in shaded soil containing clay than in shaded sandy soil. Survival was poor in sandy soils exposed either to morning or afternoon sun. Ninety days after inoculation, eggs containing motile larvae were found with difficulty or not at all in the sandy plots but were numerous, though markedly reduced, in the clay plot.

6. Due to splashing of raindrops, eggs were transported laterally at least 1.5 meters in 20 days and were deposited on vertical surfaces of boards and vegetation up to 30 cm. above the ground.

7. The sorting action of raindrops concentrated the eggs in the topmost stratum of the sandy soils but in clay they were buried under a protective layer of fine colloidal particles.

8. Low endemicity of *Ascaris lumbricoides* in the coastal plain of southeastern Georgia apparently is due chiefly to the sandy character of the topsoil which, in contrast to clay, does not contain sufficient light colloidal elements to retain surface moisture and to stratify the eggs just below the ground surface where they are protected against desiccation and direct sunlight, and at the same time are advantageously situated for transfer to new hosts.

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VARIABILITY OF *HYMENOLEPIS DIMINUTA* IN THE LABORATORY RAT AND IN THE GROUND SQUIRREL *CITELLUS LEUCURUS*

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An analysis of variability in the number and position of testes in *Hymenolepis diminuta* from naturally infected Norway rats revealed (Voge, 1952) that certain variants occurred more frequently than others, and that some types of structural abnormalities were encountered repeatedly. Examination of *Hymenolepis citelli* (McLeod, 1933), described from ground squirrels, yielded similar results. Comparison of data obtained from both species indicated clearly that a different variant was characteristic for each form.

In *H. diminuta,* three aporal testes per segment occurred most frequently; in *H. citelli* segments with one aporal and two poral testes were highest in incidence. Similarly, there was a characteristic rate of occurrence of certain structural abnormalities in *H. diminuta* which was not found in *H. citelli.* Other differences between the two species included staining reactions and proglottid shape; size was found to be similar in both forms.

Although examination of material from several different hosts yielded similar results for each species, it was of interest to determine whether the differences observed in natural infections would be maintained in cross-infection experiments. Little is known about the effects of different hosts on the development and morphology of a helminth parasite. The term "host differences" is frequently used whenever seemingly minor differences in morphology are detected in a helminth species occurring in a variety of definitive hosts. The use of this term is not desirable until more is known about the host mechanisms connected with any possible morphological changes. It is important to differentiate variability representing reaction to a particular external milieu in a particular host species from that conditioned by other factors such as heavy infection and individual variation of the helminth species not necessarily related to the host environment.

MATERIALS AND METHODS

The Rice Institute strain of *Hymenolepis diminuta* which had been kept for many years in laboratory rats, was made available through the kindness of Dr. Clark Read, Department of Zoology, University of California, Los Angeles. Cysticercoids of *H. diminuta* were reared in laboratory-bred *Tenebrio molitor.* Fourteen days after feeding onchospheres the beetles were dissected and the larval cestodes fed to two adult *Citellus leucurus.* The ground squirrels had been kept in the laboratory for several weeks prior to the beginning of the experiment and were not passing tapeworm eggs during this period. Eighteen mature *H. diminuta* were recovered from one of the ground squirrels twenty-two days after infection. Nine mature specimens of *H. diminuta* from nine laboratory rats (each infected with a single worm) were used for controls. The cestodes were fixed in Bouin's

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fluid, stained with dilute Ehrlich's haematoxylin, and treated and studied generally in a manner similar to that described by Voge (1952).

OBSERVATIONS

As noted above, it was previously reported by the author that the commonest departure from the usual position and number of testes in *H. diminuta,* from naturally infected hosts, is the occurrence of three aporal testes in each segment. The Rice Institute strain is no exception, as may be seen in Table 1 which contains data on types and frequencies of variants found in *H. diminuta* from laboratory rats and from *Citellus leucurus.* A comparison of controls and experimental worms shows that in both groups the 3a (three aporal testes) variant occurs most frequently in almost all of the specimens. The relative incidence of other variants differs somewhat in each group. This, however, is also true for different specimens of *H. diminuta* from other host species and from different strains of the same host. The

TABLE 1.—*Variability in position and number of testes in* Hymenolepis diminuta *from laboratory rats and from a laboratory infection of* Citellus leucurus

Specimen No. Rat	3a	1a 2p	3a 1p	1a 1p	2a	2a 2p	2p	3p	1a	4a	5a	No. of segments examined	Total No. variable segments	Per cent variation
1	16	3	1	3	2	0	0	0	0	0	0	298	25	8.3
2	19	5	2	4	7	1	1	0	0	0	0	430	39	9.0
3	10	2	2	1	1	0	0	0	0	0	0	390	16	4.1
4	12	4	2	4	3	0	0	0	0	0	0	420	25	5.9
5	37	2	1	2	2	3	0	0	1	1	1	664	47	7.0
6	40	3	7	4	6	0	0	0	0	0	0	534	60	11.2
7	35	6	0	1	3	0	0	0	0	0	0	396	45	11.4
8	28	7	2	4	0	2	0	0	0	0	0	465	43	9.2
9	29	2	6	6	0	0	0	0	0	0	0	510	43	8.4
Total:	226	34	23	29	24	6	1	0	1	1	1	4107	343	8.3
Citellus														
1	21	4	3	0	0	2	0	0	0	0	0	330	30	9.0
2	16	12	9	0	0	0	0	1	0	0	0	216	38	17.5
3	10	13	5	0	0	0	0	0	0	0	0	320	28	8.7
4	20	9	5	3	1	1	0	0	0	0	0	298	39	13.0
5	25	5	2	5	1	2	0	0	0	0	0	304	40	13.4
6	9	6	9	0	0	0	0	0	0	0	0	284	24	8.4
7	22	2	7	3	1	1	0	0	0	0	0	325	36	11.0
8	24	10	4	3	3	0	0	0	0	0	0	289	44	15.2
9	11	9	0	2	1	0	0	1	0	0	0	203	23	11.3
Total:	158	70	44	16	7	6	0	1	0	0	0	2569	302	11.7

usual straight line arrangement of three testes was found in the controls as well as in the specimens from *Citellus leucurus.* The environment furnished by the experimental host induced no observable changes in this regard. The staining reactions of both groups are similar in that the various organs and tissues stain with the same relative intensity. However, one finding with regard to testis variability deserves special consideration. As seen in Table 1, the total per cent variation (obtained from the total number of segments examined in each group of specimens) is lower in the controls (8.3) than in the experimentals (11.7). Since the control specimens were obtained from single worm infections, while the ground squirrel group is derived from an infection of eighteen worms in one host, one may argue that the frequency of variability may be influenced by the intensity of infection in such a manner that growth and development of the cestode will suffer certain disturbances or occur at a different rate when a large number of individuals is competing for space and food. Data in the column headed per cent variation (Table 1) indicate that the range in total per cent variation of individual specimens is consider-

ably larger in the experimental group than it is in the control group derived from single worm infections. Furthermore, the total mature proglottid count in each group shows a much larger figure for the controls. It was observed that the cestodes from these single worm infections were much larger than were those from the ground squirrel. Experiments dealing with such size differences are now in progress and will be presented elsewhere.

An examination of the abnormalities found in both groups showed that essentially the same types occurred in controls and experimental specimens. These were confluence of genital pores and ducts and incomplete separation of two otherwise normal segments. Whereas the incidence of these abnormalities was low in the control group (about five incompletely separated segments were counted), the experimental group had a higher incidence (eighteen incompletely separated segments).

The preceding observations on *H. diminuta* confirm the data recorded from natural infections of this cestode in Norway rats and also support some of the conclusions drawn by the author with respect to the differences between this species and *H. citelli* from ground squirrels. It has been suggested (Voge, 1952) that these two cestodes may be distinct species in spite of close similarity in size, and that more evidence is needed before one may safely place *H. citelli* in synonymy with *H. diminuta.* It was doubtful whether some of the differences between the two forms would be maintained in cross-infection experiments. *H. diminuta* at least remained morphologically unchanged in *Citellus leucurus,* and did not in any observable manner acquire attributes of *H. citelli* whose distinguishing characters were prevalence of certain testicular variants and abnormalities, staining intensity of some of its organs, protrusion of testes into neighboring segments, proglottid shape, and so on.

SUMMARY

Experimental introduction of *Hymenolepis diminuta* from the Norway rat into the ground squirrel *Citellus leucurus* is reported.

Variability and incidence in testis position and number, types of abnormalities and other characters of *H. diminuta* are discussed and compared in controls and experimental specimens.

The experimental specimens did not differ in any observable way from the controls.

The characteristics of both groups of specimens were similar to those of *H. diminuta* from other localities and hosts.

H. diminuta in *Citellus leucurus* did not adopt the characters which differentiate *H. citelli* from *H. diminuta.*

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THE PREVALENCE OF TOXOPLASMOSIS IN WILD PIGEONS

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It is now generally accepted that the toxoplasmas of man and of the dog, rat, mouse, rabbit, guinea pig, pigeon, and other hosts are all of the same species, *Toxoplasma gondii*. The mode of transmission of this parasite is still unknown, and it has been postulated that various animal hosts may serve as reservoirs for human infection.

The basis for such conjectures consists largely of reports on the occurrence of cases of toxoplasmosis in dogs, cats, and other animals (for review, see Callahan, Russell and Smith, 1946) and on several surveys on the prevalence of the infection in murine hosts. In the first of these surveys, Perrin, Brigham, and Pickens (1943) found 8.7 per cent of wild Norway rats in Savannah, Georgia infected. Hülphers, *et al.* (1947, *fide* Laven and Westphal, 1950) reported finding *Toxoplasma* in 27 of 840 hares in Sweden. Laven and Westphal (1950) tested serologically a total of 81 rats from 3 sections of Germany and found 10 positive. Eyles (1952) examined 90 Norway rats by inoculating 18 pairs of guinea pigs each with pooled brain tissue from 5 rats and found *Toxoplasma* infections in 5 of the pairs. Christiansen and Siim (1951) in Denmark examined histologically a large number of hares shot in a sick condition or found dead in the field. Of 2,812 animals, they found 264 or 9.4 per cent positive for toxoplasmosis.

As to birds, *Toxoplasma* has been reported from about 45 species, mostly on morphological evidence which is not always conclusive. It has been well established, however, by Carini (1911) and by Reis and Nobrega (1936) that the pigeon is a natural host of *Toxoplasma*. It was also shown by Nobrega and Reis (1942) that toxoplasmas of pigeons are capable of infecting rabbits, guinea pigs, and mice. Also, dogs and cats were infected with the same strain obtained from Reis and Nobrega by Guimaraes and Meyer (1942). In the United States, *T. gondii* was isolated in mice from the tissues of a healthy pigeon in Cincinnati, Ohio by Feldman and Sabin (1949). This pigeon had a dye test titer of 1 : 1024; two other pigeons, of 20 tested, showed titers of 1 : 64. Manwell and Drobeck (1951) found 1 of 60 pigeons from the Syracuse, New York area positive in the dye test, and on this basis postulated a 2 per cent rate for naturally occurring toxoplasmosis in these birds. Epizootics of toxoplasmosis in pigeons have been reported from Panama (Johnson, 1943), Brazil (Springer, 1942), and the Belgian Congo (Wiktor, 1950).

Our interest in pigeons as a possible reservoir of infection was aroused as a result of studies on the parasitemia in experimentally infected birds (Jacobs and Jones, 1950). Pigeons infected with the RH strain of *T. gondii* showed a high parasitemia even in the absence of acute disease. It was considered possible that pigeons with asymptomatic toxoplasmosis might serve for infecting bloodsucking arthropods in nature. Since these birds are ubiquitous and have considerable contact with man, it was deemed worthwhile to obtain information on the occurrence among them of natural infection with *T. gondii*.

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MATERIALS AND METHODS

Pigeons were trapped in the early spring of 1950 on the roof of the Capitol in Washington, D. C. They were transported to the grounds of the National Institutes of Health where they were housed in a pigeon cote with a screened fly. As space became available in the laboratory building, groups of 10 to 15 birds were removed to large cages indoors. All the birds were transferred by the summer of 1950; the majority were moved within 3 months. Thereafter, as space and time allowed, the birds were sacrificed and their tissues inoculated into mice as described below. All of the birds were sacrificed by March 1951.

The original plan called for serological testing of all the birds with the use of the *in vitro* dye test. Pigeons found positive serologically were then to be sacrificed and their tissues inoculated into mice in an attempt to isolate viable toxoplasmas. An equal number of serologically negative birds was to be selected at random and similarly tested by inoculation of mice. Fortunately, in the early stages of the investigation, a few birds were examined by inoculation of their tissues into mice before their sera were tested. Coincidentally, although the dye tests were negative, mice inoculated with suspensions of tissues from 2 of these birds became infected with *Toxoplasma.* Consequently it was decided not to place reliance on the dye test but to perform mouse inoculations with tissues from all of the birds. The results presented below are therefore derived from both serological and parasitological investigations.

Sera drawn from the pigeons were divided into 2 portions, 1 of which was inactivated at 56° C. for 30 minutes and the other kept active. Both portions were then refrigerated at −40° C. until tests were performed. In the majority of instances, only the inactivated portion was used for the dye test, but in several cases the active serum was also tested. The dye test was performed according to the method of Sabin and Feldman (1948). The sera were tested undiluted and diluted 1 : 4, 1 : 16, 1 : 64, 1 : 256, and occasionally 1 : 1024.

In the parasitological investigations, it was necessary to develop some routine which would ensure the detection of as many *Toxoplasma* infections as possible. It had been our experience, in handling a strain of *T. gondii* isolated from a dog by Perrin, that several passages in mice over an extended period of time were necessary to demonstrate the parasites. Also, in a personal communication, Dr. Harry Feldman had mentioned to us that 2 passages in mice were necessary before he succeeded in finding the toxoplasmas in histological sections of these animals subinoculated from the pigeon reported infected by Feldman and Sabin (1949), and 4 passages were made before the parasites were found in contact smears. It was therefore considered necessary to establish some routine for performing blind passages in mice inoculated with pigeon tissues. The regimen adopted was as follows: Portions of brain, liver, and spleen from the pigeon were ground in a mortar, suspended in saline, and inoculated into 2 mice, designated "O" mice. Thereafter transfers were made into fresh mice in accordance with the following schedule:

End of week										
1	1	"O"	mouse	killed	and	subinoculated	into	2	"A"	mice
2	1	"A"	"	"	"	"	"	2	"B"	"
3	1	"B"	"	"	"	"	"	2	"C"	"
4	1	"O"	"	"	"	"	"	2	"C"	"

5	1	"A"	"	"	"	"	"	2	"C"	"
6	1	"C"	"	"	"	"	"	2	"D"	"
7	1	"B"	"	"	"	"	"	2	"D"	"
8	1	"D"	"	"	"	"	"	2	"E"	"
9	1	"C"	"	"	"	"	"	2	"E"	"
10	1	"D"	"	"	"	"	"	2	"E"	"

Thus, of each pair of mice inoculated in any passage, 1 was killed and subinoculated after 1 week and the other after 2 to 4 weeks. The mice of passages C, D, and E received several inocula from mice of the preceding passages. This was done because it was not certain whether or not the optimal time for transfer of pigeon strains was early or late after infection. All inoculations were made intraperitoneally. It was recognized that a combination of intracerebral and intraperitoneal inoculations might have been more advantageous, but factors of time and sterility in a workroom devoted mainly to other projects led to the decision to use only the intraperitoneal route. The tissues of the "E" mice were examined by Giemsa-stained contact smears, when these animals were sacrificed, at the end of week 12 and week 14.

RESULTS AND DISCUSSION

Eighty pigeons were examined in this survey. The positive results are presented in Table 1. If a positive result by either or both methods of examination is accepted, 10 or 12.5 percent of the pigeons gave evidence of *Toxoplasma* infection. Only 1 of these was found positive by both methods. Of the remaining 9, 3 were found positive by mouse passage and 6 by serological tests. Thus, parasitologically a total of 4 pigeons, or 5 per cent, was found infected; serologically, 7 or 8.8 per cent gave evidence of infection.

TABLE 1.—*Positive results obtained by serological and parasitological methods in a survey of 80 pigeons for toxoplasmosis*

Pigeon No.	Dye test titer	Results of inoculations in mice
2	1 : 128	Neg.
17	Neg.	Pos. in "C" passage
19	1 : 16	Neg.
33	1 : 16	Neg.
55	1 : 16	Neg.
57	1 : 128	Pos. in "H" passage
59	Neg.	Pos. in "O" passage
64	Neg.	Pos. in "O" passage
67	1 : 1024	Neg.
78	1 : 16	Neg.

		By one or another method	By both methods	By demonstration of toxoplasmas	By dye test
Total positive	10	9	1	4	7

In one of the instances, pigeon 59, in which the bird was found positive on mouse passage but not serologically, an attempt was made to test the serum of this bird against the strain of *Toxoplasma* recovered from it. The result was completely negative, while a positive human control serum was positive in the same titer as ordinarily obtained with the RH strain of *T. gondii*. Furthermore, 2 pigeon strains, 59 and 64, were used in several dye tests with the sera of monkeys experimentally infected with RH toxoplasmas and gave results very similar to those obtained in

the same test with the RH strain. Dye tests with the active sera of these 2 birds were also negative.

It is possible to provide some explanation for the discrepancies between serological and parasitological findings at least in those cases demonstrated as positive by mouse passage. In studies on experimental toxoplasmosis in pigeons to be reported elsewhere, it has been found that birds may show only a very low or negative dye test titer 2 years after experimental infection demonstrated parasitologically. It has also been found that toxoplasmas may persist in pigeons for at least $1\frac{1}{2}$ years after inoculation. Since the birds investigated in the study reported here were all adult pigeons, it is possible that the infection may have been an old one no longer recognizable serologically.

One the other hand, the explanation for failure to find parasitological confirmation of positive serological results in this survey requires consideration of several possibilities. For one thing, the specificity of dye test titers of 1 : 16 is not certain, although as stated earlier such titers may be found in old experimental infections. The failure to find the parasite when higher dye test titers were obtained may be explainable on the basis of the existence of very few parasites in the tissues. Also, it has very recently been found in our experimental studies that in pigeons with infections of long duration, the parasites are more frequently demonstrated in the brain than in the liver and spleen. Consequently, the combination of tissues from these organs in an inoculum for mice may result in dilution of the infective organisms. Thus, parasitological findings may be negative although the organism is present in some small foci in the host. Eyles (1952) similarly found discrepancies between serological and parasitiological results in his survey of rats.

A further explanation is necessary to account for the large number of blind passages done in order to demonstrate toxoplasmas in pigeon No. 57. In observations of mice inoculated with the tissues of this bird, certain peculiarities were observed in the early passages. The mice appeared ill and showed some peritoneal exudate, but this fluid was searched in vain for toxoplasmas. Because of these peculiarities and of the positive dye test, it was decided to continue the blind passages until success was finally attained in passage "H." Apparently it required a large number of passages before the parasites became adapted to the mouse. The toxoplasmas of this strain have since been maintained routinely in mice.

The belief that the recovery of toxoplasmas from mice inoculated with tissues of a pigeon represents infection in the pigeon rather than spontaneous murine infection is based on our own observations and those of Perrin (1943). In our work with mice in this Laboratory, we have never found any instance suggestive of natural infection in these animals. Also, Perrin failed to find infection in 502 mice of the same origin although his techniques did reveal infections in guinea pigs and rabbits. Moreover, the mice used in these tests were weanlings, 4 to 5 weeks old, which would have had little opportunity to acquire toxoplasmosis even if the infection were present in the colony. The isolation of the toxoplasmas from mice after inoculation with pigeon tissues is therefore considered indicative of infection in the birds.

The significance of these findings in relation to the epidemiology of toxoplasmosis is not yet apparent. In judging the possible importance of a particular host as a reservoir of infection, it is of course necessary to have at least some knowledge of

the mechanism of transmission. This information is lacking for toxoplasmosis. Hence, it would be purely speculative to assume a connection between the infection in humans and in certain animals merely on the basis of closeness of association. In considering the dog, for example, while Westphal and Finke (1950) suggest a relationship between canine and human toxoplasmosis, they also state that they failed to find a parasitemia in experimental infections in the dog. This would indicate that dogs could not serve for infecting bloodsucking arthropods, and the hypothesis that transmission from the dog may occur by a contaminative method, while by no means ruled out, is certainly not yet proved. On the other hand, pigeons have shown a high parasitemia at least when infected with a virulent strain of *Toxoplasma*. If the possibility of transmission by arthropods is considered, the pigeon should be recognized as a likely source of infection.

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STUDIES ON THE LIFE CYCLE OF *PHYSALOPTERA HISPIDA* SCHELL (NEMATODA: SPIRUROIDEA) A PARASITE OF THE COTTON RAT (*SIGMODON HISPIDUS LITTORALIS* CHAPMAN)*

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INTRODUCTION

In the fall of 1947 two cotton rats (*Sigmodon hispidus littoralis* Chapman) were sent to the University of Illinois by the Hegener Research Supply House in Sarasota, Florida. Subsequent autopsy revealed that one animal was parasitized by nematodes of the genus *Physaloptera.* Since a complete life cycle had never been established for *Physaloptera,* such a study was undertaken.

Cram (1932) identified larvae, encysted in the breast and leg muscles of bobwhite quail and ruffed grouse, as belonging to the genus *Physaloptera.* Boughton (1937) apparently reported upon the same parasites that had been observed previously by Cram. Boughton found a cyst infection of 3.25% in ruffed grouse. The larvae were 2–3 mm. in length and were encysted near the surface of the breast and leg muscles. Alicata (1937) infected *Blattella germanica* by feeding eggs of *Physaloptera turgida* Rudolphi, a parasite of the opossum (*Didelphis virginiana*). He attempted to infect experimentally a dog, cat, rabbit, guinea pig, rat and chick with encysted third stage larvae. An autopsy was performed one month later and third stage larva were recovered from washings of the stomach of the cat and the rabbit. Third stage larvae were found encysted in the stomach wall of the rat. There was no trace of *Physaloptera* in the dog, guinea pig or chick. Hobmaier (1941) infected *Blattella germanica* by feeding eggs of *Physaloptera maxillaris* Molin, a parasite of skunks, badgers, mink and raccoon. He gave a brief description of the first and third stage larvae. Only negative results were obtained after feeding infected cockroaches to cats, dogs, and guinea pigs.

Since the writer started his studies on *Physaloptera hispida,* Petri (1950) has published on the life cycle of *Physaloptera rara* Hall and Wigdor, a parasite of dogs, cats, and coyote. He has been able to infect *Blattella germanica* and *Tribolium confusum* with this parasite. The cycle was completed by feeding cysts to, and infecting, a cat and dog. Petri and Ameel (1950) were able to infect field crickets (*Gryllus assimilis*), flour beetles (*Tribolium confusum*), and ground beetles (*Harpalus* spp.) with *Physaloptera rara.* They were also able to infect the German cockroach (*B. germanica*), camel crickets (*Centophilus sp.*) (emend. *Ceuthophilus*), and field crickets (*Gryllus assimilis*) by feeding embryonated eggs of *Physaloptera praeputialis* Linstow. Additional reference will be made to the above papers as the opportunity arises.

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* This study was begun at the University of Illinois, under the direction of Doctor Lyell J. Thomas. The writer is indebted to him and to Doctor H. Walter Steffens, of the University of Idaho, where the work was continued, for assistance and helpful suggestions. At the latter institution the work was financed by a grant from the General Research Fund.

MATERIAL AND METHODS

Most of the cotton rats used in this work were bred and reared in the laboratory. In some of the later experiments a few field-trapped rats were utilized. Trapped specimens were purchased from Hegener Research Supply House in Sarasota, Florida.

Cotton rats bred in the laboratory were weaned at 18–20 days. Whenever possible they were removed to a separate room to eliminate accidental worm infections. The gestation period for 113 progeny was 21–27 days with 3–7 rats per litter. Newborn rats are covered with hair. The eyes open 20–24 hours after birth. Rats were fed Purina dog checkers, leafy vegetables and some yellow corn. Breeding pairs were also given small quantities of raw meat.

Arthropods for infection experiments were either laboratory reared or collected in habitats where a natural spirurid infection was not likely to occur. All of the work was done outside of the natural geographic distribution of both parasite and host.

Laboratory Reared:

German cockroach (*Blattella germanica* L.)
American cockroach (*Periplaneta americana* L.)
Australian cockroach (*Periplaneta australasiae* Fabr.)
Woodroach (*Parcoblatta pennsylvanica* DeGeer)
Flour beetles (*Tribolium* sp.)

Field Collected:.

European earwig (*Forficula auricularia* L.)
Earth-boring dung beetles (*Geotrupes* sp.)
Aphodian dung beetle (*Aphodius* spp.)
Ground beetles (*Harpalus* spp.)
Sowbugs (*Armadillum* sp.)

All of the roaches, except the woodroaches, were kept in a jar of wood shavings and were fed dog checkers, and lettuce, and apple. The woodroaches were reared in jars of moist leaf mold. Flour beetles were maintained in boxes of oatmeal.

Whenever possible, the parasites were studied alive. Some specimens were fixed in warm Travassos' solution, cleared slowly in glycerine-alcohol and mounted in glycerine jelly. Material for sections was fixed in Bouin's fluid, stained with Harris' haematoxylin and counterstained with orange "G" or eosin. The salt floatation technique was used for all fecal examinations.

INFECTION EXPERIMENTS

Eggs, suspended in water, were added to small pieces of dried bread and fed to first and second instar cockroaches, and larvae and adults of the flour beetle. Such egg suspensions were added to lettuce and fed to earwigs. Ground beetles were given the eggs with raw meat. Dung beetles and sowbugs were given the egg suspension mixed with pulverized cow dung.

An examination of American cockroaches, Australian cockroaches and woodroaches, 24–48 hours after feeding eggs, revealed that many eggs had been ingested and some had hatched but all larvae appeared to be dead. Dead larvae and some unhatched eggs were passed in the feces. The dung beetles and sowbugs consumed many eggs but none were ever known to hatch. Many unhatched eggs were passed

in the feces. Larval and adult flour beetles ingested eggs and some hatched, but larvae failed to develop. In the adult flour beetles and in the American cockroaches a few amber-colored cysts were found. Such cysts contained a yellowish-brown pigment and either a dead or dying *Physaloptera* larva in an early stage of development. Alicata (1937) encountered similar cysts in *B. germanica* which were infected with *Physaloptera turgida* and *Gongylonema pulchrum.* Hobmaier (1941) and Petri (1950) mentioned similar brown cysts in connection with their work with *P. maxillaris* and *P. rara* respectively.

As a result of these feeding experiments, an infection was established in ground beetles, European earwig, and the German cockroach. The ground beetles and the earwigs were collected in the author's back yard. Approximately half of each collection was examined for a possible natural spirurid infection. All specimens were negative. Fourteen ground beetles were fed eggs of *Physaloptera hispida.* Of this number nine survived a 32-day interval for parasite development. Five of the nine contained 4, 1, 7, 2, and 2 encysted larvae respectively. Most of the larvae were fed to two laboratory-bred cotton rats. The feces of one rat were positive for *Physoloptera* eggs 80 days later. Both rats were autopsied on this date. One rat was negative while the other rat harbored three mature female and one mature male *P. hispida.*

Of the 23 earwigs that had originally been fed eggs, only 11 survived a 30-day interval for parasitic development. Six of the 11 earwigs harbored 6, 2, 9, 3, 2, and 3 larvae respectively. These larvae were fed to two laboratory-bred cotton rats. The feces of rat #1 were positive after 78 days. At autopsy the animal contained two mature female and two male *P. hispida.* Rat #2 passed the first positive feces after 84 days. It contained one mature female and three male *P. hispida.*

Large numbers of German cockroaches were infected with *P. hispida.* A total of 8 laboratory-bred cotton rats were infected by feeding encysted larvae from this intermediate host. Development of the parasite to sexual maturity in this group of cotton rats required 73–90 days. This was based in part upon appearance of eggs in the rat feces and in part, upon autopsy of three of the above rats at 65, 75, and 80-day intervals. The rat autopsied at 65 days contained only immature females.

In the German cockroach and the ground beetles the larvae developed and became encysted in the wall of the colon and rectum, although the majority occurred in the colon. In the earwigs the cysts were found only in the colon. Petri (1950) found encysted larvae of *Physaloptera rara* primarily in the rectum of the German cockroach.

It is not likely that the German cockroach functions as intermediate host under natural conditions, but earwigs and ground beetles might very well serve as possible intermediate hosts for *P. hispida* in the natural environment of the cotton rat.

Development of the Parasite in the Intermediate Host

Due to the ease with which the German cockroach can be reared in the laboratory this intermediate host was utilized for a study of the development of the larva. For this study the cockroaches were kept in a constant temperature cabinet at 28–30° C.

The eggs of *P. hispida* are embryonated when released by the female. The writer has kept one collection of eggs for eight months, at the end of which time they were still infective to the cockroach. These eggs were kept in tapwater in the

dark at room temperature. Petri (1950) found that eggs of *Physaloptera rara* remained viable for at least two months at 40° C. in 0.8% saline. Petri also mentioned that eggs of *P. rara* hatched in 0.8% saline at room temperature. The author found that viable embryonated eggs of *P. hispida* never hatched either in tapwater or saline solution; in fact, saline served to decrease the time during which eggs would remain viable.

After ingestion by the cockroach, the eggs hatch in the mesenteron and the larvae pass on to the colon where they penetrate the peritrophic membrane and invade the epithelium, causing injury to the epithelial cells. The parasite undergoes its development within this tissue.

At the time of hatching, the larva (Fig. 1) measures 0.164–0.183 mm. in length and 0.0097–0.0109 mm. in width. There is a prominent tooth at the anterior end. Other structures are not visible at this time. By the 4th day a large renette cell develops. This opens ventrally through a pore in the anterior third of the larva. At this time the esophagus and at least the anterior half of the intestine are visible. By the seventh day the genital primordium appears (Fig. 17) about midway of the intestine. By the 13th day the larva attains a length of 0.311–0.317 mm. and a width of 0.025–0.029 mm. About this time (Fig. 6) the cuticle loosens in preparation for the first molt which occurs at 14–17 days. The structures of the second stage larva are visible through the loosened cuticle.

The initial stages in the formation of a cyst are coincident with the first molt. For details of encystment and the host tissue reactions see Schell (1952). It will be sufficient to mention here that the cyst membrane is definitely a product of the host, arising as a result of extensive fibrosis in the colon wall. By the 20th day the cyst can be dissected from the colon wall as a separate spherical structure. At this stage the membrane is delicate and ruptures easily. A cyst may enclose more than one larva. As development progresses the cyst protrudes more and more on the outer surface of the colon wall and, in some instances, may become pedunculate, attached to the colon by a thin stalk.

The second stage larva (Fig. 2) grows rapidly. The esophagus becomes less bulbous at the posterior end and between esophagus and intestine several distinct cells appear. The larva has two lateral lips, each with a V-shaped suture. Behind the lips are several large cells, possibly arcade cells. The renette cell is now more compact with a slender duct. At 16–18 days the genital primordium is composed of 8–12 cells, but sexual differentiation is still not evident. The second molt occurs at 24–27 days. The anterior and posterior ends of a larva about to undergo a second molt are shown in Figures 8 and 10.

The third stage larva (Figs. 3 and 4) has two triangular lips, each with a sharp lateral tooth and one subdorsal and a subventral oral papilla. The esophagus now consist of a narrow anterior portion and a longer and wider posterior region. The intestine grows more rapidly than the rest of the larva and is thrown into folds (Fig. 16). Sexes can now be distinguished. The female genital primordium (Fig. 11) is sac-shaped and is attached by its anterior end to the ventral body wall. It tends to be located farther forward than the male genital primordium (Fig. 12) which is bi-lobed. In larvae that have completed their growth in the intermediate host, the male primordium is 0.48–0.56 mm., whereas the female primordium is 0.5–0.68 mm. from the posterior tip of the larva. The writer could not detect any consistent

difference in size between male and femal larvae at this stage. The third stage larva completes its growth 30–35 days from the time of hatching. By this time it attains a length of 1.04–1.2 mm. and a width of .08–.1 mm.

Alicata (1937) examined three German cockroaches 14 days after feeding eggs and found first and second stage larvae of *Physaloptera turgida,* "free in the body cavity." On the 27th day he examined two more cockroaches and found third stage larvae encysted in the tissues surrounding the body cavity. This indicates that there is a penetration of the digestive tract followed by encystment somewhere in the haemocoel. The writer has studied larval development of *P. hispida* and *P. turgida* in the German cockroach. Observations from many dissections and sectioned material reveals that the larva does not penetrate the digestive tract of the cockroach and never gains access to the haemocoel. The larvae develop within the tissues of the colon or the rectum within a cyst membrane. This same condition prevails in ground beetles and earwigs. Encystment is a gradual and complex process involving a cellular response by the host. (cf. Schell 1952)

Petri (1950) detected the earliest indication of a first molt in *P. rara* at 11 days after exposure to eggs; however, he seemed to regard the 16th day as a more normal time for this molt. Third stage larvae were first found 21 days after feeding eggs. Petri never found larvae free in the body cavity.

Development in the Definitive Host

Eleven laboratory-bred cotton rats were infected with *P. hispida* and used for a study of the development of the parasite in the definitive host. These rats were autopsied at chosen intervals in order to determine the rate and site of development. The parasite undergoes all of its development in the pyloric region of the stomach. The larvae feed separately during the first 30–40 days with their lips imbedded in the stomach mucosa, causing scattered inflamed areas. Following this period they tend to congregate and feed in a compact group (Fig. 9). Continued feeding in this manner eventually results in the formation of a chronic ulcer.

The larvae grow rapidly in the cotton rat, attaining a length of 6–8 mm. within the first 14–18 days. The genital primordia of male and female larvae, after 16 days in the definitive host, are shown in Figures 14 and 15. By the 25th day the larvae attain a length of 14–17 mm. The growth rate varies somewhat in different cotton rats. Between 60 and 65 days males and females can be found in copulation. In all of the cotton rats infected in the laboratory the parasites developed to sexual maturity, depositing embryonated eggs in the feces within 73–90 days. The parasites continue to grow for sometime after attaining sexual maturity. Fully developed females attain a length of 53–64 mm. and a width of 1.4–1.9 mm. Males reach a length of 30–42 mm. and a width of 0.9–1.4 mm. Petri and Ameel (1950) found mature specimens of *P. rara* in a kitten 83 days after feeding encysted larvae.

Pathology in the Definitive Host

Seurat (1937) illustrated a "cupule" produced by *Physaloptera getula* Seurat in the stomach of *Meriones shawi* Rozet in North Africa. Yokagawa (1922) briefly described a "tumor" caused by *P. formosana.* Hoeppli and Feng (1931) studied the effects of secretions from the glandular esophagus of *P. clausa* upon bacteria

and tissues of various rodents. They were unable to demonstrate the liquefaction of tissues or a bactericidal property of esophageal secretions.

In the cotton rat the continued feeding by the parasites in a compact group results in the formation of a chronic ulcer which may attain a diameter of 10 mm. The older the infection and the more parasites present, the larger the ulcer tends to become. On the outer surface of the stomach the ulcerated area appears as a hard, white ring. On its inner surface the ulcer is circular with an elevated margin and a sunken inflamed central area in which the worms do their feeding. A section through a 7 mm. ulcer is shown in Figure 19. In it the mucosa and submucosa are completely destroyed. The muscularis always remains intact. In the center there is extensive infiltration by polymorphonuclear leucocytes. At the base of the ulcer there is considerable fibrosis resulting in a walling-off of the inflamed area. The margin of the ulcer exhibits marked hyperplasia. Infected cotton rats seem to exhibit no noticeable external symptoms.

Infective Second Stage Larvae

This work was begun by feeding second stage larvae, 19 and 20 days old, to two laboratory-bred cotton rats. Due to the thinness of the cyst membrane at this stage of development, many of the larvae escaped from the cyst. After a number of negative fecal examinations and an interval of 101 days, the above animals were autopsied. They contained 26 and 8 immature *Physaloptera* respectively. The parasites had attained approximately half of their normal growth. In each rat, an ulcer, 5.–6. mm. in diameter, had formed. The worms were of uniform size and sexes were approximately equal. Later a third rat was fed second stage larvae. This animal was autopsied after 122 days. The parasites in this instance were about two-thirds grown.

It appears that the cotton rat can become infected by ingesting second-stage larvae. The possibility of a molt in the egg was considered and effort was made to detect such a molt. All attempts were unsuccessful. Two larval molts had been previously observed in the intermediate host.

In order to learn what happened to second-stage larvae after ingestion, and also to find some reason for the retarded development, encysted second-stage larvae 20–22 days old were fed to a series of nine field-trapped cotton rats (40–60 cysts per rat). These rats had been in the animal room for at least five months. Fecal examinations were negative for spirurid eggs. Autopsies were made at intervals of 1 (two rats); 3; 7; 11; 17 and 19 (two rats) days after feeding the encysted larvae. It was found that within 24 hours the cyst membrane is lost (possibly digested), and the second-stage larva attaches loosely to the stomach wall and starts feeding. Instead of molting on the 24–27th day of larval life (or 2–4 days after ingestion of cysts), as would normally occur in the intermediate host, the second molt is delayed approximately 13 days. Third-stage larvae were first encountered in the cotton rat examined on the 17th day. Third stage larvae were also found in the two rats examined on the 19th day. The delayed molt accounts for part of the retarded development but not all of it. This study was not continued.

Two cotton rats were given repeated feedings of a considerable number of first-stage larvae, but no infections were established.

Two albino rats and, later, two cotton rats were fed many embryonated eggs. Results were negative.

Infection of Other Rodent Hosts

Encysted larvae of *P. hispida* were fed to two deer mice (*Peromyscus sp.*), two Norway rats (*Rattus norvegicus*), two rice rats (*Oryzomys palustris natator*), and two albino rats. All of these specimens had been live-trapped except the albino rats. The rice rats were from Sarasota, Florida. The deer mice and Norway rats were trapped in the vicinity of Champaign, Illinois. Prior to feeding cysts, the feces of all animals were negative for spirurid eggs.

One deer mouse died after 34 days. It was examined and found to be negative for *Physaloptera.* The second deer mouse was autopsied after 54 days but contained no *Physaloptera.* The rice rats were autopsied after 93 days but were negative. The Norway rats were autopsied on the 25th day. The stomachs of these rats contained two and ten immature *Physaloptera,* respectively. One albino rat was autopsied on the 54th day. It harbored 11 immature *Physaloptera.* The other albino rat was examined at 85 days and five mature specimens (3 male and 2 female) *P. hispida* were recovered. The feces of this rat contained *Physaloptera* eggs on the day of autopsy.

Superinfections

Two cotton rats were given successive feedings of encysted larvae at chosen intervals in order to determine whether superinfections could be established. The parasites recovered were of three distinct sizes and are designated as larvae; immature, and mature specimens. The data in Table I indicate that the worm population can build up as a result of repeated infections.

Rat no.	Feeding dates	Age of infection in days	Parasites recovered
52	11–23–48	151	3 (mature)
	2–20–49	62	5 (immature)
	4– 5–49	18	14 (larvae)
55	1–20–49	93	2 (mature)
	3– 9–49	45	4 (immature)
	4– 1–49	22	12 (larvae)

SUMMARY

Physaloptera hispida Schell is a parasite of the cotton rat (*Sigmodon hispidus littoralis* Chapman). A life cycle is established for the parasite with *Forficula auricularia, Harpalus* spp. and *Blattella germanica* serving experimentally as intermediate hosts.

In the intermediate hosts the larvae develop in the wall of the colon or rectum, causing local injury to host tissues. The larva undergoes two molts and is enveloped by a cyst membrane while in the intermediate host. A fully developed third stage larva is produced in 30–35 days. The parasite attains sexual maturity in 73–90 days in the definitive host. Cotton rats were infected by ingesting second stage larvae, but in such infections the development of the parasite is retarded. Superinfection can be established in the cotton rat.

P. hispida will also develop in the albino rat and the Norway rat. In the definitive host the feeding activities of the parasite cause the formation of a chronic ulcer, which exhibits fibrosis, extensive leucocyte infiltration, induration, and hyperplasia.

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Plate I

All illustrations were made from camera lucida drawings except Figure 9.

Fig. 1. Larva hatching from egg.
Fig. 2. Second-stage larva, 16 days.
Fig. 3. Tail of third-stage larva, 35 days.
Fig. 4. Third-stage larva, anterior end.
Fig. 5. Embryonated egg.
Fig. 6. Larva with loosened cuticle, about to undergo first molt, 13 days.
Fig. 7. Colon of German cockroach with 4 cysts, each containing one third-stage larva.
Fig. 8. Anterior end of larva undergoing second molt, 24 days.

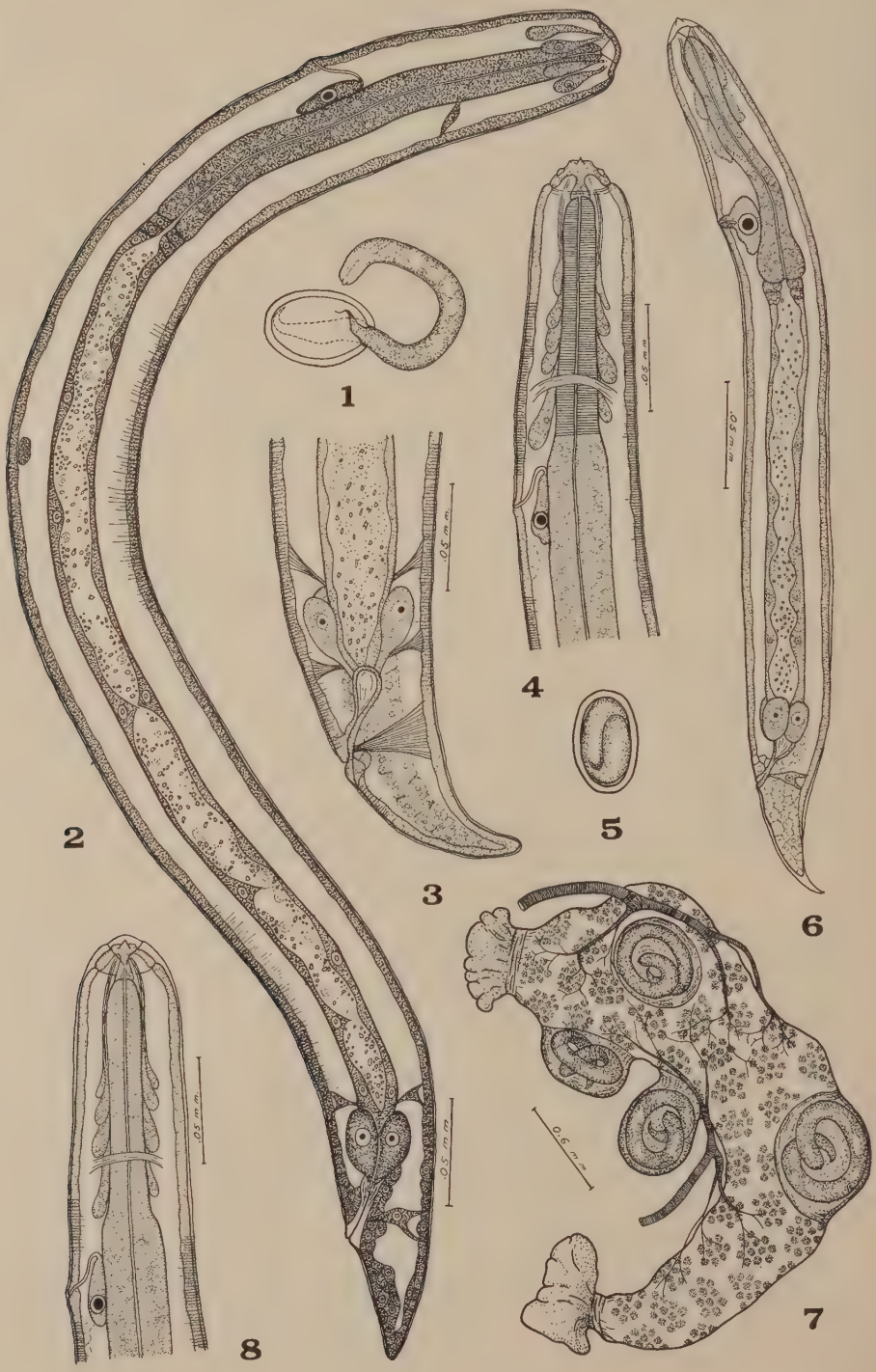
Plate II

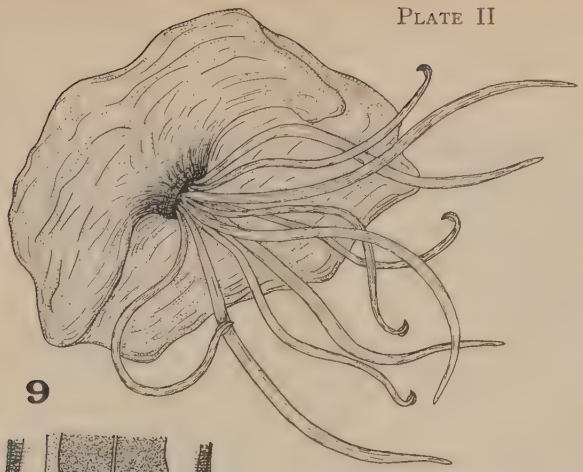
Fig. 9. Adult parasites feeding at an ulcer.
Fig. 10. Tail of larva during second molt, 24 days.
Fig. 11. Female genital primordium, 25 days.
Fig. 12. Male genital primordium, third-stage larva.
Fig. 13. Anterior end of third-stage larva, ventral view, showing lips and papillae.
Fig. 14. Male genital primordium, 18 days in cotton rat.
Fig. 15. Female genital primordium, 18 days in cotton rat.
Fig. 16. Encysted third-stage larva, 31 days.
Fig. 17. First-stage larva, 7 days, showing renette cell and single-celled genital primordium.

Plate III

Fig. 18. Sectioned adult parasite in feeding position.
Fig. 19. Section through a 7 mm. ulcer.

PLATE I





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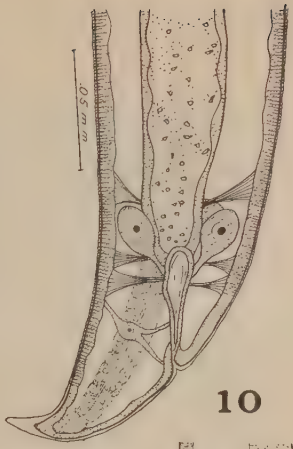

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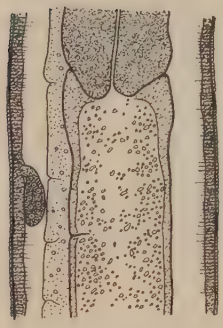

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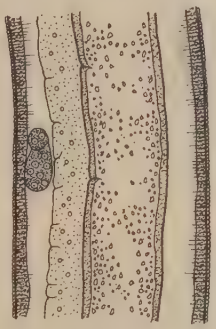

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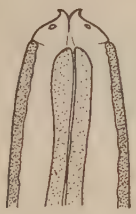

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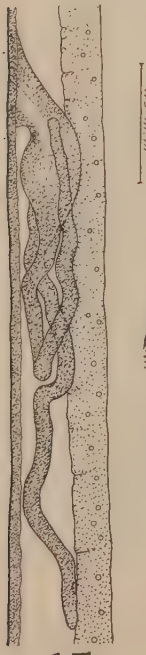

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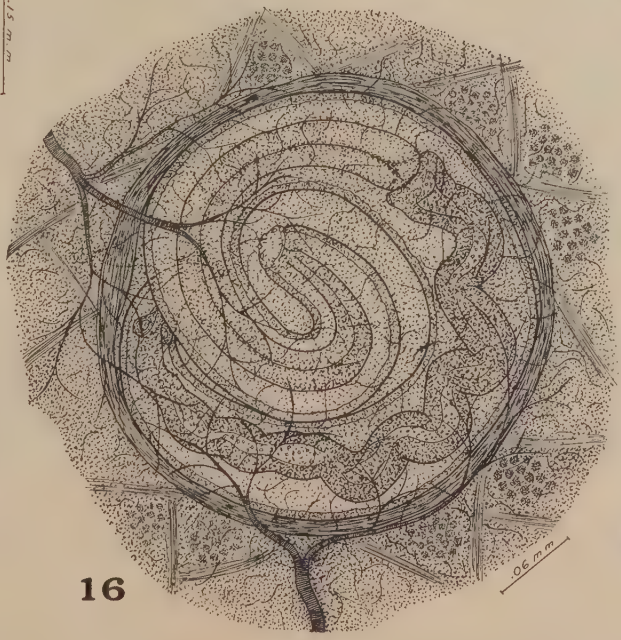

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PLATE III

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OBSERVATIONS ON THE BIOLOGY OF THE ARGASID TICK, *ORNITHODOROS BRASILIENSIS* ARAGÃO, 1923, WITH THE RECOVERY OF A SPIROCHETE, *BORRELIA BRASILIENSIS*, N. SP.

GORDON E. DAVIS[1]

In March 1951, 31 specimens of *Ornithodoros brasiliensis* were received from Prof. Henrique de B. Aragão, Instituto Oswaldo Cruz, Rio de Janeiro. They had been collected by Prof. Raul di Primio from the soil in São Francisco de Paula, Rio Grande do Sul, Brazil. Locally, this tick is known as the dog tick, and as the "Mouro bug." It is found in the soil under houses inhabitated by man, or shelters for domestic animals. It has also been taken from the dens of skunks, *Conepatus* sp.

The lot was composed of six females, three males, and 22 nymphs in varying stages of development. When allowed to feed individually on white mice they engorged with amazing rapidity, but there was a tendency to remain attached for some time after engorgement, during which time a copious amount of coxal fluid was passed on the host.

The present study concerns: (1) the rearing of ticks from the larval to the adult stage, and (2) the recovery of a spirochete which causes relapses in white mice. Primio (1934a, b) was unable to obtain progeny from ticks of this species. Although relapsing fever has not been reported from the State of Rio Grande do Sul, Pinto and Primio (1931) reported that following tick bite there is at times headache, dyspnea and a rise in temperature, and they speculated on the possible relation of the tick to the disease.

REARING OF TICKS

The six females mated and three produced eggs. None of the eggs from one female hatched. There were three larvae from the second, and 110 from the third.

The Larvae:

The larvae were placed on the clipped belly of a white mouse. They were very inactive, but after a time 15 attached and took small amounts of blood. None became fully distended. Five of these died without molting, and the molting range for the remaining ten was from 7 to 28 days. Fifty-one additional larvae died without molting or feeding. About 10 days after the first attempt at feeding it was found that all of the surviving unfed larvae had molted to the first nymphal stage. Although a few of the ticks fed in the larval stage, it appears that this is not the rule and that the molting of the unfed larvae to the first nymphal stage after a period of days constitutes a fourth molting and feeding pattern for ticks of this genus. These patterns are as follows:

1. Alternate feeding and molting from the larval to the adult stage, e.g., *O. turicata, O. hermsi, O. parkeri, O. rudis, O. tholozani, O. erraticus,* etc.
2. The larvae feed and then there are two successive molts without further feed-

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ing. These larvae require several days for engorgement, e.g., *O. talaje, O. puertoricensis, O. coriaceus, O. dugesi,* etc.

3. The eggs hatch and the larvae molt to the first nymphal stage almost simultaneously. The two known species which exhibit this pattern are *O. moubata* and *O. savignyi.*

4. The eggs hatch and in about 10 days the unfed larvae molt to the first nymphal stage, e.g., *O. brasiliensis.*

Following the first feeding, the ticks under study were placed individually in serially numbered cotton-stoppered vials and stored at room temperature in a closed jar over a saturated solution of ammonium chloride which maintains a relative humidity of 79 ± per cent.

Molting:

After each molt, the ticks were fed individually and then returned to their respective vials. Daily observations were made for molting. The tabulation is as follows:

Stage	Molting range in days	Number of ticks	Adults
Larval	7–28	15	
1st nymphal	8–10	51	
2nd "	12–34	51	
3rd "	17–68	49	
4th "	20–63	48	9 males, 2 females
5th "	20–34	37	9 males, 13 females
6th "	20–45	13	12 females
7th "	40	1	1 female

The Nymphs:

In the first nymphal stage, molting was very regular in from 8 to 10 days, but the range became wider in later nymphal stages. In numerous other species of the genus the ticks are not ready to feed for some days after molting, but throughout the nymphal stages of *O. brasiliensis* the ticks fed readily within 48 hours after molting. As presented above, from four to seven nymphal stages were required before the adults emerged. We have observed six nymphal stages in *O. coriaceus,* and Robinson (1946) has reported the same number for *O. delanoei acinus* which is the largest number of nymphal stages previously reported.

The Adults:

The first adult was a male which emerged 103 days after the initial feeding. The first female emerged in 120 days, and the last female, after seven nymphal stages, emerged in 184 days.

Of the 46 ticks reared to the adult stage, 18 were males and 28 were females. Twenty of these females have engorged and mated from one to three times without oviposition. One was placed in its native Brazilian soil in which it was received, but it still failed to oviposit. The original female from which the 46 ticks were reared has fed and mated repeatedly, but has failed to oviposit a second time. The females feed frequently, although seemingly fully distended. This is in contrast to the females of many other species which refuse to feed over a period of months unless oviposition has taken place.

THE RECOVERY OF A SPIROCHETE

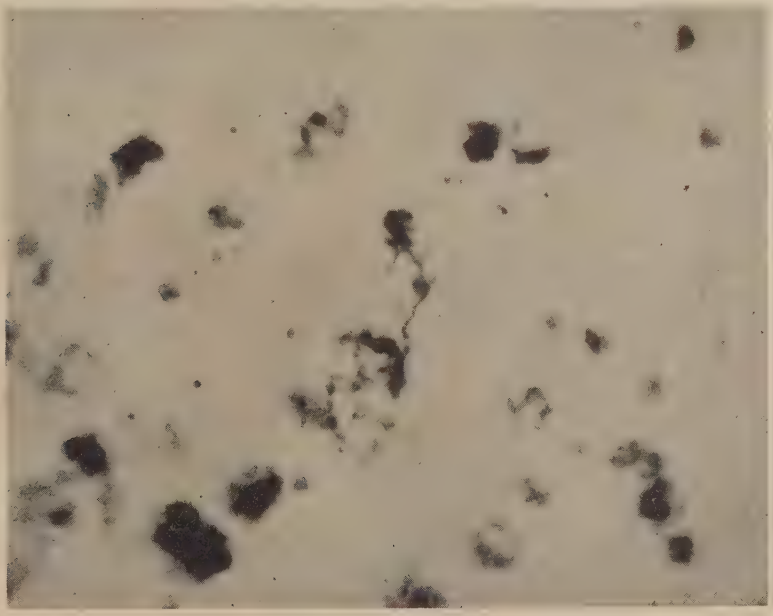
On the sixth day following the first test feeding of one of the original nymphs, a spirochete was recovered from the blood of the host mouse. The organisms were

very scarce. They appeared again on the tenth and eleventh days. Transfers were made by tail blood with the results noted in table 1.

TABLE 1.—Borrelia brasiliensis *n. sp. in white mice.*

Passage	Mouse numbers	Days spirochetes present in peripheral blood	Remarks
1st	19028	3rd, 4th, 6th, 7th, 8th, 10th	Negative to 25th day
	19029	2nd, 3rd, 8th	"
2nd	19037	2nd, 3rd, 13th, 14th	"
	19038	2nd, 3rd, 8th, 9th, 14th	"
3rd	19104	5th, 6th, 12th	Negative to 21st day
	19105	5th, 6th, 10th	"
4th	19106	3rd, 4th, 11th, 14th	"
	19107	3rd, 7th, 8th, 17th	"
5th	19283	5th, 7th, 15th	"
	19284	3rd, 5th, 7th, 8th, 13th	"
6th	19416	3rd, 4th, 8th	"
	19417	3rd	"
7th	19418	Negative	"
	19419	4th	"

After the seventh passage the strain was lost and has not been recovered again from the original tick. Meanwhile, two guinea pigs had been injected with infective mouse blood. Spirochetes appeared in the peripheral blood of one guinea pig on the sixth and seventh days, and in the blood of the other on the fifth and sixth days. There was one day of fever in each animal, 40.5 C. and 40.1 C., respectively.



Borrelia brasiliensis n. sp. in blood of a white mouse.

Although there is little, morphologically, to distinguish this spirochete from other species, as may be seen from the accompanying photograph made from the original isolation, the tick vector is new and the locality is far removed geographically from areas where spirochetes are known to be present in other species of *Ornitho-*

doros. As thus far reported, there has been a marked degree of specificity between ticks and their respective spirochetes in the Western Hemisphere. It was on this basis that *B. turicatae, B. hermsii,* and *B. parkeri* were named.

The name *Borrelia brasiliensis* n. sp., is therefore proposed for the spirochete normally transmitted by *O. brasiliensis.*

SUMMARY

1. Forty-six ticks, the progeny of one female *O. brasiliensis,* were reared to the adult stage. This constitutes the first rearing of ticks of this species.

2. The molting and feeding pattern constitutes a fourth type, in that unfed larvae molt to the first nymphal stage in about 10 days after hatching.

3. There were from four to seven nymphal stages before the adults emerged.

4. Throughout the nymphal stages the ticks feed readily within 48 hours after molting.

5. A spirochete which causes relapses in adult white mice was recovered. The term *Borrelia brasiliensis* n. sp. is proposed for this spirochete normally transmitted by *O. brasiliensis.*

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BIOLOGY AS AN AID TO THE IDENTIFICATION OF TWO CLOSELY RELATED SPECIES OF TICKS OF THE GENUS *ORNITHODOROS*

GORDON E. DAVIS[1]

Recent studies on several lots of ticks from California, identified on a morphologic basis as *Ornithodoros turicata,* but shown biologically to be *O. parkeri* cast doubt on some of the published records for *O. turicata* from this state.

The type specimens of *O. turicata* (Dugès), 1876, were described from Guanajuato, Mexico; and the type specimens of *O. parkeri* Cooley, 1936, were described from Wyoming, U.S.A. Geographically, *O. turicata* has a southern distribution while *O. parkeri* has a northern distribution. Morphologically, the two species differ mainly in the size and distribution of the mammillae and in the relative lengths of the hypostomes (Cooley and Kohls, 1944). In the laboratory they interbreed and produce fertile progeny (Davis, 1942), but otherwise they differ widely, biologically.

As listed by Cooley and Kohls (1944), *O. turicata* was first recorded from California by Banks from San Diego County in 1908. Further listings by these authors are from Riverside County, Harbinson (1935); Madera County, Kelley (1939); and Alameda County, Holdenried (1940).

An adult tick found on a man in Merced County was described as *O. wheeleri* n. sp. by McIvor (1937). Hoping to obtain more specimens of this new species, the writer collected a large number of ticks from Kern and Fresno Counties which were identified as *O. turicata.* Following biologic studies, which indicated that the ticks were *O. parkeri* and not *O. turicata,* a further morphologic study was made by Dr. R. A. Cooley and Dr. Barbara C. McIvor of undoubted *O. turicata* and *O. parkeri;* of the California specimens collected by the writer; and of the newly named *O. wheeleri;* and it was agreed that all ticks from the area were *O. parkeri.* These ticks were then listed as *O. parkeri,* and *O. wheeleri* was placed in synonymy (Cooley and Kohls, 1944).

In 1943, the writer collected ticks in the same area as Holdenried's 1940 collection. These ticks were also identified as *O. turicata* by Dr. R. A. Cooley.

In 1948, specimens received from Yolo County were identified as *O. parkeri* by Glen M. Kohls of this Laboratory. These ticks were then studied biologically and the results confirmed the identification. This study was based on only one of the criteria to be mentioned. The tests were as follows:

Ten of the California ticks, and ten *O. parkeri* (Washington stock) engorged on a white mouse infected with an Idaho *O. parkeri* strain of spirochetes. The ticks were subsequently tested for transmission by feeding individually on white mice. There was 60 per cent transmission by the California ticks and 90 per cent transmission by the Washington *O. parkeri.* Similarly, ten of the California ticks and ten *O. turicata* (New Mexico stock) engorged on a mouse infected with *O. turicata*

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spirochetes. When tested as above there was no transmission by the California ticks, but there was 100 per cent transmission by *O. turicata.*

Meanwhile, Burroughs and Holdenried (1944) collected ticks in Kern and Alameda Counties, which were identified as *O. turicata,* and they recovered relapsing fever spirochetes from the ticks. This was the first report of *O. turicata* as a potential vector in California. Finally, these ticks were sent to the writer. In an attempt to maintain the spirochetes, which were readily recovered from the ticks, it was noted that they had certain characteristics of *O. parkeri* spirochetes in that they were more tenuous than *O. turicata* spirochetes, and stained less deeply by Giemsa's method. This observation prompted a further investigation on a biologic basis.

The biologic criteria, thus far established for the differentiation of *O. parkeri* and *O. turicata,* are: 1. Each species of tick transmits its own spirochete but fails to transmit the spirochete normally transmitted by the other (Davis, 1942).

2. *O. turicata* may transmit spirochetes through the eggs up to 100 per cent (Davis, 1943), while transovarial transmission in *O. parkeri* has never been demonstrated.

3. *O. turicata* from the U.S.A. has regularly failed in the transmission of the spotted fever rickettsias (Brumpt, 1936a, 1936b), (Brumpt and Desportes, 1941), (Davis, 1939), while *O. parkeri* transmits these agents by feeding, and also through the eggs to at least the fourth generation (Davis, 1943).

4. In *O. parkeri* there is a rest period during the winter and early spring months (Davis, 1941), and oviposition may be very long delayed, while *O. turicata* is active throughout the year.

The ticks from Kern and Alameda Counties, identified on a morphologic basis as *O. turicata,* were therefore tested according to these criteria.

1. Transmission of spirochetes: A mouse was infected by feeding one of the California ticks which had been identified as *O. turicata.* When spirochetes appeared in the peripheral blood, ten *O. turicata* (New Mexico stock) and ten *O. parkeri* (Montana stock) were allowed to engorge on the mouse. These ticks were subsequently tested for transmission by feeding individually on white mice. There was 70 per cent transmission by *O. parkeri* and none by *O. turicata.*

2. Transovarial transmission: A female from Kern County was shown to be infective on December 22, 1949, and again on February 2, 1950. Oviposition took place the latter part of April. Fifty-nine of the progeny were tested in the larval stage, 35 of which survived the larval molt, in the first nymphal stage; and 32, which survived the first nymphal molt, in the second nymphal stage. None of the 126 mice which served as hosts to these ticks became infected.

3. Transmission of spotted fever rickettsias: Several experiments were initiated, but for the most part the results were rendered valueless by early death of the guinea pigs as a result of intercurrent infections. However, one experiment provided the required evidence to establish this point. Ten ticks, in the first nymphal stage, from the Alameda County stock, engorged on a guinea pig infected with spotted fever. Following the first test feeding there was a febrile period and scrotal swelling in the guinea pig, which was subsequently shown to be immune when challenged with blood which produced typical symptoms in control guinea pigs.

4. Inactive periods—delayed oviposition: Two females engorged, and mated with males of the same lot August 23, 1951, and again on January 14, 1952. Neither female has oviposited to the present date (April 1952).

During this period, numerous *O. turicata* females have oviposited regularly. Two extreme records of delayed oviposition in *O. parkeri* are 15 months, and two years, respectively, after the last feeding.

SUMMARY AND CONCLUSIONS

Ticks from California which had been identified on a morphologic basis as *O. turicata,* and one lot which had been identified on the same basis as *O. parkeri,* have been subjected to the established biologic criteria for differentiation of these species. The *O. parkeri* were tested according to the first of the following criteria, and the *"O. turicata"* were tested according to the four. These criteria are: (1) the specific relationship which exists between the two species of ticks and their respective relapsing fever spirochetes; (2) the regular transovarial transmission of the spirochete in *O. turicata,* with a complete failure of such transmission in *O. parkeri;* (3) transmission of the spotted fever rickettsias by *O. parkeri* but not by *O. turicata;* and (4) an inactive period for *O. parkeri* throughout the winter months, while *O. turicata* remains active during this period.

The identification of one lot of ticks as *O. parkeri* was confirmed by the fulfillment of criterion 1. The remaining ticks identified on a morphologic basis as *O. turicata* were shown to be *O. parkeri* by the fulfillment of the four criteria established for this species.

It is therefore concluded that the ticks from Alameda and Kern Counties are *O. parkeri* and not *O. turicata,* contrary to previous reports, and further, that *O. turicata* has not thus far been shown to be a potential vector of relapsing fever spirochetes in California.

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HELMINTHS OF NORTHWEST MAMMALS. PART III. THE DESCRIPTION OF *EURYHELMIS PACIFICUS* N. SP., AND NOTES ON ITS LIFE CYCLE[1]

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During the spring and summer of 1951, one of the authors (C.M.S.) and Kenneth A. Neiland had the opportunity to make a preliminary survey of the helminths of some Oregon mammals. A trematode of unusual interest was collected from the mink, *Mustela vision* Schreber, the muskrat, *Ondatra zibethica* (Linnaeus), and the water shrew, *Sorex bendirii palmeri* taken near Portland, Oregon. This fluke had the general anatomy and egg-size characteristic of *Euryhelmis squamula* (Rudolphi 1819) and occurred with this species in the mink and the shrew, but it differed from it in shape. At first the worms were considered to be immature specimens of *E. squamula* but further study has shown that its life cycle involves a salamander rather than a frog. Because of these and other differences this fluke is considered a distinct species for which the specific name *E. pacificus* is proposed. Certain peculiarities of this species also require emendation of the genus.

All of the material from the mink, the muskrat, and the shrew was fixed in formalin-alcohol-acetic acid solution, stained with Semichon's acetocarmine with a counterstain of fast green, cleared in either xylene or terpineol, and mounted in Clarite. The experimentally reared material was handled as above or was fixed with either corrosive-acetic acid solution or Bouin's fluid and stained with Ehrlich's hematoxylin. Serial cross, sagittal, and frontal sections of several thicknesses also were made. These were stained with Galigher's alum hematoxylin and eosin. The measurements appearing in the specific diagnosis were taken from twenty-one specimens from the mink and muskrat.

We wish to thank the Oregon State Game Commission and trappers Lee and Homer Taylor for their very kind assistance in obtaining the mink and muskrat utilized in this study and Dr. Robert Rausch who graciously furnished slides of *Euryhelmis monorchis* for comparison.

Euryhelmis pacificus n. sp.

(Figs. 1–5)

Specific diagnosis: Euryhelmis. Body thin, leaflike, transparent, spinose over-all, pyriform or elongate. Length 0.66 to 1.04 mm.; width 0.34 to 0.68 mm. Oral sucker appearing either terminal or subterminal 0.035 to 0.087 mm. long by 0.049 to 0.090 mm. in diameter. Pharynx large, spherical, 0.035 to 0.059 mm. in diameter, connected to the oral sucker by a short but definite prepharynx from 0.004 to 0.100 mm. but averaging 0.039 mm. Esophagus slender, bifurcating anterior to the acetabulum. The intestinal ceca extend obliquely to the sides, then follow the contour of the body to the posterior end where they almost touch in some cases. Ventral sucker 0.035–0.062 mm. in diameter, situated slightly pre-equatorially. Two testes ovoid or lobed, opposite or oblique in the posterior region of the body. Right testis usually more flattened anterio-posteriorly and wider transversely than left testis. Right testis 0.18–0.32 mm. by 0.10–0.16 mm. Left testis 0.15–0.26 mm. by 0.14–0.18 mm. Large sac-like seminal vesicle dorsal to uterus and posterior and to the right of acetabulum, constricted into a spherical posterior cham-

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[1] This work was supported by contract Nonr-15300, NR 132-992, between the Office of Naval Research, Department of the Navy, and Reed College.

ber and an elongate anterior chamber, being connected to genital atrium by a short ejaculatory canal. No copulatory organ. Genital atrium located immediately anterior to the acetabulum and overhung by a bilobed gonotyl. Gonotyl which appears to be a fold of tissue rather than a sucker, averages 0.050 to 0.014 mm. Uterus consisting of three or four loops confined between intestinal ceca, testes, and acetabulum, opens into genital atrium to the left of ejaculatory canal. Ovary, located on right side anterior to right testis, generally club-shaped, 0.10 to 0.25 mm. by 0.07 to 0.14 mm. Seminal receptacle, located between right testis and ovary, spherical or club-shaped 0.07 to 0.23 mm. by 0.06 to 0.16 mm. Laurer's canal originates as a medial elongation of seminal receptacle and, after some coiling, opens on the median dorsal surface somewhat posterior to oötype region. Mehlis' gland well developed, located to the left of ovary. Vitelline follicles numerous, confined laterally, extending from near the bifurcation of the intestinal ceca to the posterior end. Eggs operculated, 0.020 to 0.034 mm. by 0.010 to 0.017 mm. in preserved material, 0.031 to 0.014 mm. in fresh material. Excretory bladder Y or T-shaped, extending forward from the posterior end of the body between the testes and bifurcating immediately anterior to them.

Hosts: Mustela vision Schreber, *Ondatra zibethica* (Linnaeus) and (experimental) white rat, golden hamster, and the field mouse *Peromyscus maniculatus* (Wagner); adults without eggs in *Sorex bendirii palmeri.*

Habitat: Small intestine.

Location: Oregon, U. S. A.

Type specimen: U. S. Nat. Mus. Hel. Coll. No. 47830; paratypes, Dept. of Biology, Reed College, and authors' collection.

Euryhelmis Poche, 1926 diagnosis emend.

Generic diagnosis: Heterophyiidae. Body small, flattened, leaflike. Excretory bladder Y or T-shaped. Testes, one transitory or two, spherical or lobate, in the posterior half of the body. Cirrus and cirrus pouch absent. Seminal vesicle present or absent. Uterus with only an ascending limb, relatively short, confined between the intestinal ceca. Vitelline follicles numerous, mainly laterial, extending from near the intestinal bifurcation to the posterior region of the body. Oral sucker, acetabulum, pharynx, and esophagus present. Prepharynx present or absent. Intestinal ceca long, extending to the posterior extremity of the body. Genital atrium immediately anterior to the acetabulum, overhung by a bilobed, fold-like gonotyl. Eggs operculated, with or without slight polar thickening.

Type species: Euryhelmis squamula (Rudolphi 1819)

Euryhelmis pacificus differs from *E. squamula* and *E. monorchis* Ameel, 1938, in being characteristically pyriform in shape, in having the oral sucker larger than the acetabulum, in having a definite prepharynx, and in having a salamander for a second intermediate host. It further differs from *E. monorchis* in possessing two non-transitory testes.

NOTES ON THE LIFE CYCLE

Sperm: The sperm are long slender structures measuring about 0.2 mm. in length and less than 0.001 mm. in width.

Eggs: The eggs are rather thick-shelled, operculated, with a polar thickening which is not spine-like. Each egg appears to contain a fertilized ovum and five or six yolk cells. The prominent operculum is set in a collar and could not be removed by rolling or pressure.

The snail host and the cercaria are as yet unknown.

Metacercaria: The Pacific Giant Salamander, *Dicamptodon ensatus* (Eschscholtz) is the only species found infected by the metacercaria of *E. pacificus* in this area. The larval form of this salamander is quite abundant in many of the rocky streams of the Coast and Cascade Range in Oregon. In all, about fifty larvae and three adults have been collected by the authors from eight widely separated points. The only two specimens which were taken from the Coast Range in Tillamook County harbored no metacercariae. However, about ninety per cent of those col-

lected from five localities in the Cascade Range were infected. An adult found on the Reed College Campus also was very heavily infected. Salamanders more than 50 mm. long were nearly always infected. None of the several hundred other amphibians collected in the same areas harbored this metacercaria. However, *Rana aurora* (Baird and Girard) and *Rana cascadae* Slater, 1939 were found to be infected with a metacercaria tentatively identified as *E. squamula.*

The number of metacercariae per host varied from five or six to several hundred but usually was around fifty. The cysts were located in the striated muscles of the host and not in the subcutaneous connective tissue where, in frogs, the cysts of *E. squamula* and *E. monorchis* are found. The cercariae appeared to have no preference as to the part of the host in which they encyst as the metacercariae were rather evenly distributed. The cysts, 0.15 to 0.29 mm. were surrounded by a yellowish loose connective tissue capsule formed by the host. This capsule measured 0.21 to 0.40 mm. in diameter, and was easily located in the translucent striated muscle. The cyst wall was 0.0015 mm. thick, transparent and tough. The metacercariae are apparently well adapted to their host as they seemed to remain as viable in salamanders kept alive in the laboratory for five months as in freshly collected specimens.

When excysted the metacercariae were quite active and showed well developed reproductive organs. Stained and mounted specimens average 0.680 mm. long by 0.350 mm. wide. The oral sucker is 0.061 to 0.071 mm. The pharynx, 0.039 mm. in diameter is connected to the oral sucker by a definite prepharynx averaging 0.024 mm. The acetabulum averages 0.049 mm. in diameter. The intestinal ceca of many of the excysted metacercariae were packed with discrete bodies the exact nature of which has not been determined. The main excretory canal was also distended to a lesser degree with discrete droplets which presumably were respiratory by-products. The gonads and genital ducts were easily seen in living metacercaria, the testes were spherical and were not yet producing sperm; the ovary was oval or club-shaped. The seminal receptacle which had a definite spherical shape, was lined with cilia as were the oviduct, Laurer's canal, and the duct leading to the ootype. These cilia could be seen to beat actively in living specimens. The primordia of the uterus and seminal vesicle did not appear to be lined with cilia.

Adult: A young white rat from our laboratory stock was fed about fifty of the metacercaria. When the rat was examined six days later, forty-eight mature *E. pacificus* were found in the duodenum. Later, about a dozen white rats were fed these metacercariae and examined after varying lengths of time. All of them were infected with *E. pacificus.* In infections of less than forty-eight hours, the flukes contained no eggs and very few or no sperm were found in the seminal receptacle, while in infections of sixty hours or more a few to many eggs and large numbers of sperm were present. It appears therefore that in the white rat, the flukes must mature about fifty-five hours after infection and persist for about eight months. In the golden hamster the flukes were mature seventy-two hours after infection.

A wild field mouse, *Peromyscus maniculatus,* which was fed metacercariae seven, six, and one day before examination, harbored ten flukes of which only half contained eggs and these had less than six eggs each. Two white mice fed metacercariae three and seven days before examination apparently harbored no flukes. Since the four specimens of *E. pacificus* found in the water-shrew were devoid of

eggs, it appears that these small mammals do not serve as satisfactory, natural definitive hosts.

Incidence and host relationship. Two of eleven mink examined during the 1950–51 trapping season were found to be infected with *E. pacificus.* These two mink, which were also infected with *E. squamula,* were taken on the upper Sandy River whereas the uninfected ones came from the lower Columbia River area. Of thirty-four muskrats taken during this same period, only one was found to be infected with *E. pacificus.* This muskrat was taken on the Reed College campus while all of the uninfected ones came from the Columbia River ponds near Portland. The infected animals each harbored over a hundred of these flukes. These figures do not, of course, give a true picture of the situation especially in the case of the mink, as the salamander intermediate host is not abundant along the lower Columbia River. A much higher rate of infection in the mountainous areas around Portland is indicated by a high rate of metacercarial infections in the salamander from these regions.

The presence of this infection of the muskrat is quite interesting since the muskrat is generally not considered to be carnivorous in its feeding habits. It appears that they must eat amphibians under some conditions and thereby infect themselves.

Variations. Of 293 specimens of *E. pacificus* examined, 11 or 3.7% were mirror images of the more typical form, that is had the ovary, seminal receptacle and seminal vesicle on the left side, 1, or 0.3%, had only one testis, and 4, or 1.3%, showed a degeneration of the vitelline follicles in the posterior region. Of 108 specimens of *E. squamula* 5 or 4.6% were mirror images and 1, or 0.9%, showed a degeneration of the vitelline follicles.

Remarks. The genus *Euryhelmis* was proposed by Poche (1926) for the fluke *Distomum squamula* (Rudolphi 1819) which was poorly described. For this reason Witenberg (1929) did not include the genus in the family HETEROPHYIIDAE. A complete description of *Euryhelmis squamula* was published by Baer (1931) and Callot (1946) published measurements of adults. Ameel (1938) described a new species, *E. monorchis,* with an abbreviated report of its life cycle. Price (1940) in the light of recent studies of life cycles reviewed the superfamily OPISTHORCHIOIDEA, placing the genus *Euryhelmis* in the subfamily APOPHALLINAE Ciurea 1924 of the family HETEROPHIDAE. The authors are in accord with this position. The typical heterophyid shape of *E. pacificus* and the position of the second intermediate host between the typical fish host of most heterophyids and the frog host of the other species of *Euryhelmis* suggest that this form may be a link between the ancestral heterophyid and the present *E. squamula* and *E. monorchis.*

Summary. A new species of HETEROPHYIDAE, *Euryhelmis pacificus,* from mink and muskrat is described and notes on the life cycle are given. The second intermediate host is *Dicamptodon ensatus.* Incidence, distribution, and variation are mentioned.

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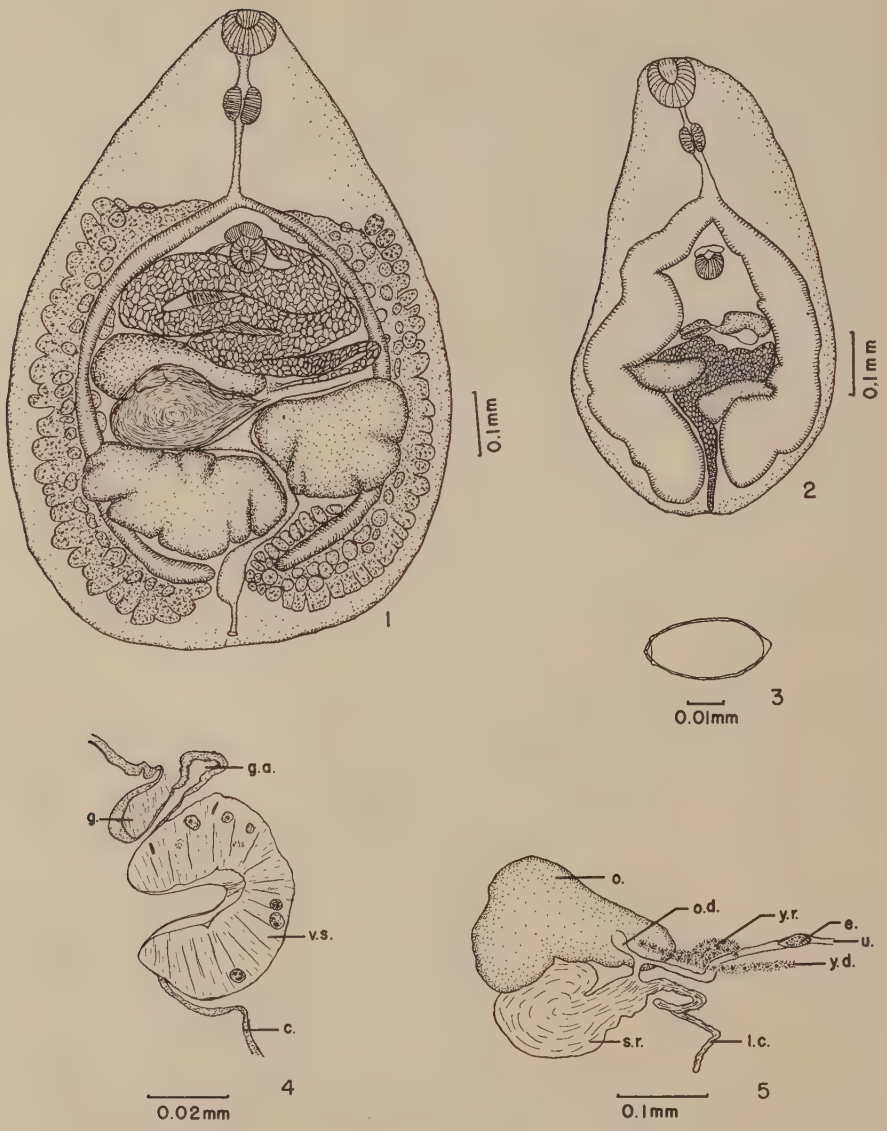
PLATE I



EXPLANATION OF PLATE

FIG. 1. Adult *E. pacificus* n. sp. (ventral view).
FIG. 2. Metacercaria excysted (dorsal view).
FIG. 3. Egg.
FIG. 4. Ventral sucker and gonotyl, longitudinal, cut at 3 μ.
FIG. 5. Oötype region (ventral view).

Explanation of Lettering in Plate: c, cuticle; e, egg; g, gonotyl, ga, genital atrium; lc, Laurer's canal; o, ovary; od, oviduct; sr, seminal receptacle; u, uterus; vs, ventral sucker; yd, vitelline duct; yr, vitelline reservoir.

A NEW SPECIES OF MICROSPORIDIA FROM THE FAWN-COLORED LAWN MOTH, *CRAMBUS BONIFATELLUS* (HULST) (LEPIDOPTERA, CRAMBIDAE)[1]

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In the fall of 1948 the Laboratory of Insect Pathology of the Division of Biological Control at the University of California began a study of the possibility of using entomogenous microorganisms in the control of sod webworms. To obtain insects for laboratory testing, adults of the fawn-colored lawn moth, *Crambus bonifatellus* (Hulst), were collected in the Berkeley, California, area and an insectary population was established.

Shortly after this work was started a heavy mortality was noted in the laboratory-reared insects. The diseased larvae and pupae were found to contain a microsporidian. The spores appeared to have a wide size-range varying from 4 to 8 microns in length. Many of the smaller spores were grouped together in clusters of eight indicating the possibility that two species of Microsporidia may be present. A similar situation was encountered by Steinhaus and Hughes (1949) in their studies of Microsporidia parasitizing the potato tuberworm, *Gnorimoschema operculella* (Zeller). Experimental transmission of the spores to larvae of the buckeye caterpillar, *Junonia coenia* Hübner, and to other hosts resulted in successful isolation of a microsporidian with spores varying in length from 5 to 8 microns. These large spores are never found in clusters and the microsporidian is considered to belong to the genus *Nosema* Naegeli. The eight-spored microsporidian never has been isolated and is considered to be an unidentified species of the genus *Thelohania* Henneguy because of the grouping of the spores. The purpose of the present paper is to report the study on the life history of the isolated species of *Nosema*.

Nosema infesta n. sp.

Life Cycle

After oral ingestion by the host, the spores appear to undergo a pregermination development in which the two sporoplasm nuclei migrate to the anterior end of the well-strained sporoplasm (Fig. 1 R). This phenomenon has been reported previously in the life cycle of *Perezia pyraustae* Paillot (Hall, 1952).

Upon germination, the entire sporoplasm leaves the spore through the anterior pore (Fig. 1 S). The sporoplasm readily stains blue with Giemsa's solution and characteristically has a single large nucleus at the anterior end (Fig. 1 A). It has the same appearance as the sporoplasm within the spore prior to germination. A typical specimen measures about 4 microns in length and tapers in size from 2 microns at the posterior end to 1 micron at the anterior end. Although no irregularly appearing specimens were seen, it is assumed that this form corresponds to the amoebula stage of other descriptions. The sporoplasm rounds out to an oval or

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[1] Contribution from the Laboratory of Insect Pathology, Division of Biological Control, College of Agriculture, University of California, Berkeley.

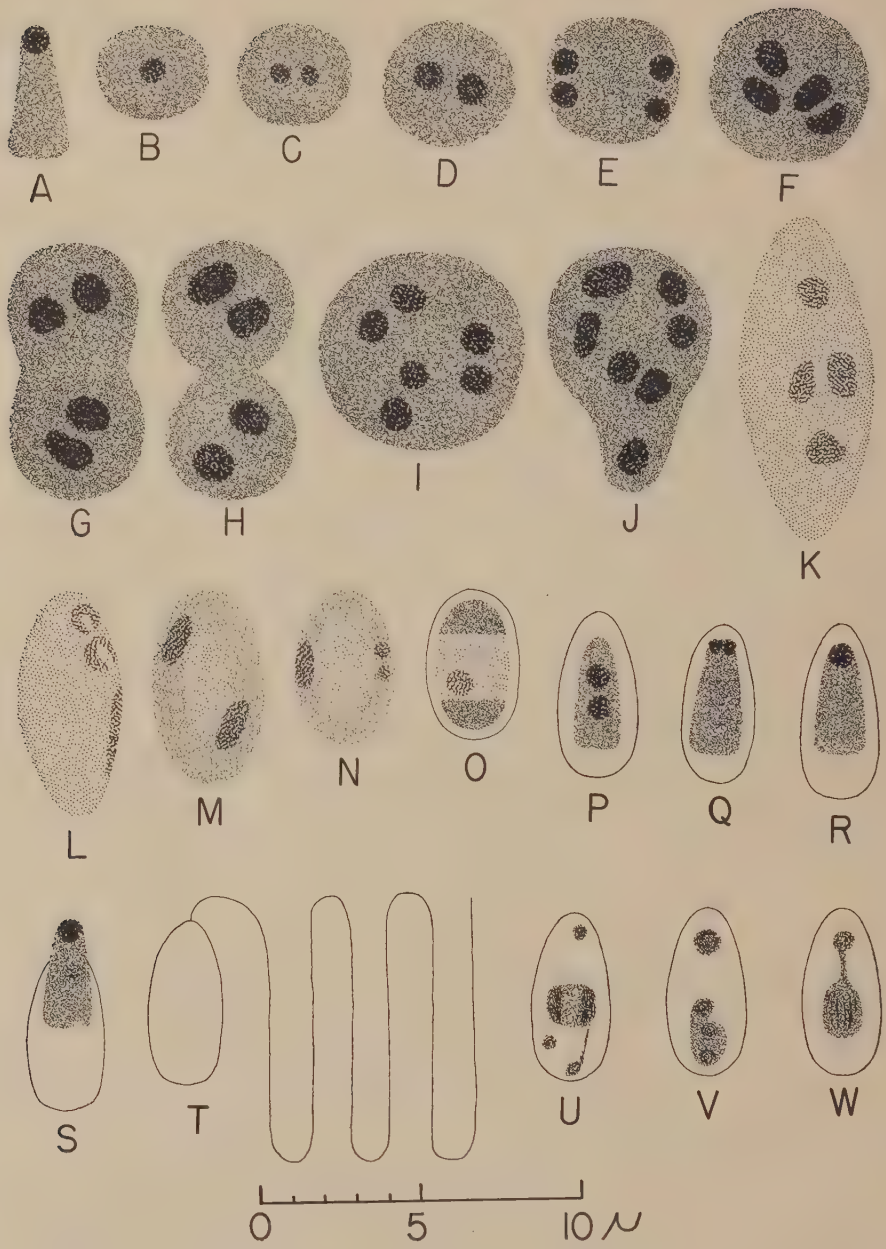

FIG. 1. Various stages in the life history of *Nosema infesta* n. sp. A. Emerged sporoplasm. B–J. Schizonts. K–L. Sporonts. M–N. Sporoblasts. O. Immature spore. P–R. Spores. S. Emerging sporoplasm. T. Extruded polar filament. U–W. Unusual spores in buckeye caterpillar.

globular shape and very soon the nucleus moves to the center of the cytoplasm and divides, giving a binucleate condition (Fig. 1 B, C, D).

The typical schizont is binucleate, although forms with one, three, or four nuclei (Fig. 1 D–H) are quite frequent. More rarely seen are forms with six to eight nuclei (Fig. 1 I, J). The nuclei vary in size and shape, probably depending upon the size and shape of the schizont body. With Giemsa's stain, the nuclei of the younger schizonts stain a bright red while the dense cytoplasm is a uniform blue. The cytoplasm of the older forms stains more unevenly. In the smaller schizonts the nuclei are more compact while in the larger forms the nuclei are more diffused. Occasionally the nuclei display lightly staining centers. In some cases, the nuclei

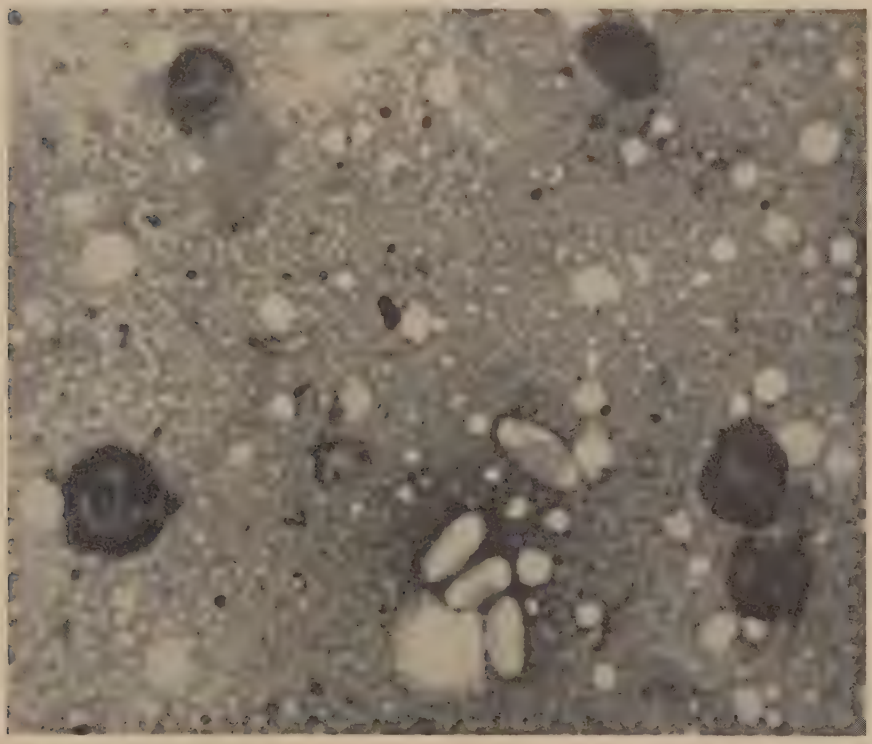

FIG. 2. Vegetative and spore stages of *Nosema infesta* n. sp.

are surrounded by a clear area in the cytoplasm. Binary fission has been seen in the binucleate and tetranucleate forms (Fig. 1 G, H). The 6- to 8-nucleate forms appear to divide by budding into uninucleate or binucleate forms (Fig. 1 J). Frequently seen are older schizonts with compact nuclei grouped in pairs or singly along the periphery of the cell. The schizonts range in size from that of the small rounded sporoplasm to as much as 12 microns in diameter. They are generally rounded in form although irregular shapes are often seen.

The sporont stage must be of rather short duration since very few are seen in smear preparations. It is an elongate form measuring from 5 to 10 microns in length and 3 to 5 microns in width (Fig. 1 K, L). The cytoplasm is highly irregular

in density and stains very lightly. From 2 to 4 nuclei have been seen, indicating that the sporont stage may evolve from either the binucleate or tetranucleate schizonts. More commonly, the nuclear material is scattered throughout the cytoplasm in irregular clumps.

What is thought to be the sporoblast stage is an elongate structure with rounded ends. It varies from 5 to 8 microns in length and is about 3 microns in width. The cytoplasm is concentrated in the peripheral region in some forms, since with Giemsa's solution the middle region stains a very light blue while the outer region is a darker blue (Fig. 1 M, N). Two or four clumps of nuclear material have been seen in the regions of dense cytoplasm along the periphery. In later forms, the cytoplasm appears to have drawn away from the ends of the structure and condensed in the central region. The nuclear material appears to concentrate into several small solid masses.

In the young spore, the cytoplasm stains a uniform grey-blue and often two nuclei can be seen in the sporoplasm (Fig. 1 P). The nuclei usually lie along the longitudinal axis of the spore. As the spore matures, the wall thickens and the cytoplasm and nuclei stain more lightly. The spores vary in length from 5 to 8 microns and in width from 2 to 3 microns. Polar filaments have been seen by observing with darkfield illumination wet mounts of spores which have been subjected to pressure (Fig. 1 T). The longest filament observed measured 120 microns. Shorter filaments of from 50 to 70 microns were more common. No swollen distal ends were observed.

Examination of unstained spore suspensions obtained from experimental infection of the buckeye caterpillar revealed the presence of occasional spores in which the spore walls were very transparent and the spore contents were a bright red color (Fig. 1 U–W). Most of the red-colored spores had one large irregular clump of material in the posterior half of the spore and a smaller clump of material at the anterior end. In some spores, several small clumps of material were seen. In many cases, strands of red-colored material connected the clumps together. What phenomenon this red spore represents is unknown, but it is of interest that they were found only in material obtained from transmission through larvae of the buckeye caterpillar. No red-colored spores were found in material obtained from the sod webworm or the California oakworm, *Phryganidia californica* Packard.

In late stages of infection when many spores are in evidence, most of the spores stain very poorly, but there are always a few spores that stain easily and appear to be going through the previously mentioned pregermination development. This would indicate that this microsporidian is capable of reinfecting tissues of the same host by repetition of development. The principal seat of infection is the adipose tissue.

Since each sporont forms a single sporoblast which in turn forms a single spore, the microsporidian under consideration must belong to the genus *Nosema* Naegeli. This microsporidian may be distinguished from most binucleate species of *Nosema* since it does not form schizont chains containing more than two cells. Other differentiating characters are the size and shape of the spore, position of the nuclei within the spore, and the length of the extruded polar filament. Comparison of the microsporidian with the descriptions of other binucleate species (Kudo, 1924, 1947; Paillot, 1939; Steinhaus and Hughes, 1949) leaves little doubt that it is a new spe-

cies. Accordingly, the name *Nosema infesta* n. sp. is proposed for it (*infesta*—from the Latin *infestus* meaning hostile, unfriendly).

Although *Nosema infesta* was first observed in laboratory-reared sod webworms, it is not known if the fawn-colored lawn moth is the principal natural host. Almost 100 per cent infection by the microsporidian has been observed in larvae of *Crambus bonifatellus* and the fiery skipper, *Hylephila phylaeus* Drury, that were collected from lawns in El Cerrito, California. Experimentally, the larvae of a number of other insects are known to be susceptible to oral infection by *Nosema infesta.* These include: California oakworm, *Phryganidia californica* Packard; alfalfa caterpillar, *Colias philodice eurytheme* Boisduval; imported cabbageworm, *Pieris rapae* (Linnaeus); larvae of the malva butterfly, *Vanessa carye* (Hübner); buckeye caterpillar, *Junonia coenia* Hübner; salt-marsh caterpillar, *Estigmene acraea* (Drury); potato tuberworm, *Gnorimoschema operculella* (Zeller); beet webworm, *Loxostege sticticalis* (Linnaeus); granulate cutworm, *Feltia subterranea* (Fabricius); beet armyworm, *Laphygma exigua* (Hübner); and the armyworm, *Cirphis unipuncta* (Haworth).

SUMMARY

A new species of Microsporidia, observed in laboratory-reared larvae of the fawn-colored lawn moth, *Crambus bonifatellus* (Hulst) is described. The name *Nosema infesta* n. sp. is proposed for it. In addition, data relating to natural and experimental host range are given.

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RESEARCH NOTES

A COMPARISON OF THE INFECTIVITY OF *SCHISTOSOMA JAPONICUM* OCCURRING IN JAPAN FOR *ONCOMELANIA NOSOPHORA* AND *ONCOMELANIA FORMOSANA*

Schistosoma mansoni from distinct endemic centers varies in its infectivity for specific intermediate snail hosts; furthermore, a single species of snail occurring in several centers may vary in susceptibility. These distinctions were referred to as physiological, or strain diffrences, by Files and Cram (1949, J. Parasit. 35 (6) : 555–560), who clearly presented the interrelationships of four strains of *S. mansoni* and three species of snail from five areas. Their observations were substantiated by previous and contemporary investigations (Vogel, 1942, Zentralbl. Bakt. 148: 29–35; Stunkard, 1946, J. Parasit. 32: 539–552; Cram, et al., 1947, Nat. Inst. Health Bull. 189: 81–94; Abdel-Malek, 1950, Am. J. Trop. Med. 30 (6) : 887–894). An initial experiment has been made to determine if such differences occur in relation to *Schistosoma japonicum* and two of its intermediate hosts, *Oncomelania nosophora* and *O. formosana.* Both snails were exposed to *S. japonicum* autochthonous to Japan.

Specimens of both snail species were exposed simultaneously to miracidia of a single hatching. Exposures were made by (1) placing a single snail in a few drops of water containing either one or ten miracidia, or (2) by dropping a single miracidium into the shell aperture; the latter was least effective. Snails exposed to ten miracidia were dissected before the mother sporocysts lost their identity, making it possible to determine the infectivity of the miracidia. When snails were exposed to single miracidia, time was allowed for infections to reach maturity.* The results of the experiments are shown in the following table:

SUMMARY OF INFECTION EXPERIMENTS WITH MIRACIDIA OF *Schistosoma Japonicum*

	O. formosana	*O. nosophora*
Exposures with One Miracidium		
Snails Exposed	200	200
Snails Surviving	194	128
No. Infected*	0	12
Per cent Infected	0.0	9.4
Exposures with Ten Miracidia		
Snails Exposed	135	135
Snails Surviving	126	117
No. Infected	1	52
Per cent Infected	0.8	44.4
Total Miracidia	1260	1170
Mother Sporocysts		
No. Recovered	1	77
Recovery Rate**	0.08	6.6

* Determined by shedding 5 months after exposure; a 10% sample was crushed.
** Ratio of mother sporocysts to total miracidia used.

Of 128 surviving *O. nosophora* exposed to one miracidium, 12 or 9.4% were infected, while not a single infection occurred among 194 *O. formosana.* When each snail was exposed to ten miracidia, 52 or 44.4% of 117 surviving *O. nosophora* were infected with a total of 77 mother sporocysts; the latter figure represented 6.6% of the total miracidia used. In contrast, only one of 126 *O. formosana* was infected following exposure to ten miracidia. This single infection was probably an experimental one, as a preliminary check by sample crushing had been made, and the infection was of the same age as those in *O. nosophora.* The question remains as to whether the infection could have reached maturity.

It appears that *O. formosana* is an unsuitable host for autochthonous Japanese infections. Yet, since it serves successfully as a host of *S. japonicum* in Formosa, there must be a strain difference between *S. japonicum* of the two countries.—GEORGE W. HUNTER, III, L. S. RITCHIE AND Y. OTORI, *406th Medical General Laboratory, APO 500, c/o P.M., San Francisco, California.*

GLYCOGEN CONSUMPTION IN ACANTHOCEPHALA UNDER AEROBIC AND ANAEROBIC CONDITIONS

Preliminary experiments have been conducted to compare the aerobic and anaerobic glycogen consumption under conditions of starvation in the thorny-headed worm of hogs, *Macracanthorhynchus hirudinaceus*. This was considered essential as a basis for further studies on the carbohydrate metabolism of these parasites. For each experiment about thirty adult female worms were collected from the intestines of freshly slaughtered hogs and placed in a Ringer-Tyrode solution without glucose. The worms were washed several times in this solution and divided into three groups and weighed. The glycogen content of the control group was determined immediately, and the other two groups of worms were placed individually in 125 cc. Erlenmeyer flasks containing about 100 cc. of solution to which penicillin and streptomycin had been added (140 mg. of penicillin, crystalline G, and 50 mg. of dihydrostreptomycin sulfate to 1 liter of medium). These two groups were kept in an incubator at 37 degrees C. for 43 hours. One of these latter groups was kept under aerobic conditions by leaving the flasks unstoppered and bubbling oxygen through the medium every 6–8 hours. The other group was kept under anaerobic conditions by using boiled medium, replacing the air in the flasks with nitrogen, and leaving tightly stoppered. At the end of 43 hours incubation the worms were usually all alive and there was no visible growth of micro-organisms in the medium. It has been suggested that streptomycin interferes with aerobic respiration in bacteria, and it is possible that this might also occur in the worms, but this seems unlikely with the concentration used in view of the fact that the worms remained alive and appeared to be in good condition throughout the experiment. Fairbairn and Reesal (*Science* 112: 792, 1950) reported the use of streptomycin in experiments with *Ascaris;* they observed no ill effects on the worms.

TABLE 1.—*Aerobic and anaerobic glycogen consumption of* Macracanthorhynchus hirudinaceus *females under conditions of starvation at 37 degrees C.*

Experiment No.	At beginning of experiment			After 43 hours under aerobic conditions				After 43 hours under anaerobic conditions			
	No. worms	Weight of worms in gms.	Glycogen in per cent of fresh weight	No. worms	Weight of worms in gms.	Glycogen in per cent of fresh weight	Glycogen consumption in gms. per 100 gms.	No. worms	Weight of worms in gms.	Glycogen in per cent of fresh weight	Glycogen consumption in gms. per 100 gms.
1	10	25.990	2.31	5	17.253	0.97	1.34	5	10.900	0.29	2.02
2	10	38.654	2.13	10	33.631	0.65	1.48	5	18.623	0.35	1.78
3	5	16.774	2.38	5	10.626	1.13	1.25	5	11.180	0.90	1.48
4	10	20.097	1.38	10	32.004	0.42	0.96	10	32.036	0.34	1.04
5	9	25.152	1.08	10	29.109	0.62	0.46	10	27.914	0.39	0.69

The thorny-headed worms kept under aerobic conditions seemed to be in a slightly better condition than the others as evidenced by the fact that they moved spontaneously while those kept under anaerobic conditions moved only when stimulated by pinching with forceps. The pH of the medium at the end of each experiment, as estimated by the use of pHydrion papers, was only slightly more acid than at the beginning, and no difference between the aerobic and anaerobic flasks was noted; it appears that acid production is not a major factor. It is possible that the buffer in the medium was strong enough to prevent any significant change in pH. The glycogen was determined by the method of Good, Kramer and Somogyi (J. Biol. Chem. 100: 485, 1933) after freezing the worms with carbon dioxide. The glycogen was hydrolyzed with normal HCl and the glucose determined by the method of Somogyi (J. Biol. Chem. 160: 61, 1945). Readings were taken with a Klett-Summerson photoelectric colorimeter using appropriate standards for comparison. The results are summarized in Table I; the glycogen is expressed as glucose in per cent of the fresh weight of the worms.

Von Brand (J. Parasit. 26: 301, 1940) found the glycogen content of *Macracanthorhynchus* females to be 1.35 and 1.16 per cent of fresh weight in two sets of experiments. The higher figure (1.86, average at beginning) obtained by the writer might be due to differences in technique or possibly to differences in the food of hogs serving as hosts. The average glycogen consumption over a period of 43 hours was 1.09 grams per 100 grams of worms under aerobic conditions and 1.40 under anaerobic conditions. The quotient of the anaerobic : aerobic glycogen consumption is therefore 1.27. Von Brand (l. c.) studied the aerobic glycogen consumption of *Macracanthorhynchus* and found that the worms consumed 0.8 gram per 100 grams of worms over a period of 23 hours. Using the data of Weinland and Rudolph (1910), whose experiments were believed to be anaerobic, he estimated a quotient of 1.2 for a 24 hour period. In experi-

ments with *Ascaris lumbricoides* Von Brand (Ztschr. vergleich. Physiol. 21: 220, 1934) found a quotient of 1.2. In free-living forms, however, this quotient is much higher because the complete oxidation of carbohydrate results in the liberation of much more energy than anaerobic decomposition of a corresponding amount of carbohydrate. The relatively low quotient in the intestinal helminths indicates that anaerobic processes predominate even under aerobic conditions. The explanation for this is not clear; possibly there is a lack of the necessary enzyme systems to permit complete oxidations.

The intermediate products of carbohydrate metabolism have not been investigated in the Acanthocephala. Von Brand (l. c.) found that the major products of carbohydrate metabolism in *Ascaris lumbricoides* were volatile fatty acids, chiefly valeric acid, while in the tapeworm, *Moniezia expansa,* he found (Ztschr. vergleich. Physiol. 18: 562, 1933) that the major products were higher fatty acids, succinic acid and lactic acid. It would be of interest to learn whether *Macracanthorhynchus* resembles *Ascaris* or *Moniezia,* or whether it forms still other intermediate products. The writer wishes to express her indebtedness to Dr. S. R. Tipton for valuable suggestions in carrying out these experiments.—Helen L. Ward, *The University of Tennessee, Knoxville.*

A METHOD OF MAKING WHOLE MOUNTS OF MOSQUITO LARVAE FOR SPECIAL STUDY

During the course of a recent study of chaetotaxy of culicine mosquito larvae, the author had occasion to examine approximately 500 *Culex* (*Melanoconion*) larvae mounted individually or in small groups in various media, prepared in various laboratories in the western hemisphere, and ranging in age from several days to about 40 years. As this rather detailed investigation progressed, it became evident that some revision of mounting technique was necessary, inasmuch as a high proportion of the specimens were virtually useless for a detailed analysis. In the first place, many of the specimens were very dark when mounted, or had turned dark in storage, preventing critical observation of sclerotized internal body parts or of fine ventral hairs under a high dry objective. Moreover, most of the mounting media used in the oldest of these slides had become so clouded that an ordinary microscope could not resolve minute structure.

The present investigation was carried out to obtain larvae transparent enough so that subsequent darkening was impossible, so that any possible alterations in the mounting medium would not visibly affect the observation of minute structure, and so that the proper amount of contrast in the cleared specimen would be retained. Accordingly, several systems of preparation were tested, including graded series of alcohols and the use of various clearing and mounting media. The regimen proposed below is believed to be the most satisfactory synthesis of these trials.

Procedure: 1. Drop living larvae into a beaker of water at 180 degrees F. and leave for approximately 10 seconds, or until the body has acquired a definite whitish cast.

2. Remove specimen with a wide-mouth pipette, place directly in 10 per cent KOH at room temperature until the body becomes transparent. During the first hour or two the body will shrivel and become nearly black, but continued treatment with KOH will cause the thorax and abdomen to swell to their original proportions.

3. Wash for one-half hour in distilled water, allow to drain, and place in fresh glacial acetic acid for 24 hours. There is no visible shrinkage in this step.

4. Transfer directly to white Cellosolve (ethylene glycol mono-ethyl ether), where it should be left for not less than two hours.

5. Transfer the larva to three volumes of fresh Cellosolve and add one volume of beechwood creosote. In two hours this dilution may be decreased by adding another volume of creosote, so that the proportion of Cellosolve to creosote is now 3 : 2. In another two hours add a third volume of creosote to bring the proportions to 1 : 1.

6. Further dilution of the Cellosolve is not necessary, and the larva is transferred directly to a drop of thin balsam on a slide and coverslipped.

For this particular study a mount was desired which would retain its rotundity so that the hairs could be observed in their original life-like positions with respect to each other. This was accomplished by placing the larva in a pool of balsam held in a 7/16-inch hole drilled through a piece of 1/64-inch celluloid which had previously been cemented to a slide with balsam. This hole was slightly smaller than the 15 mm. round coverslip which was completely supported by the celluloid around its perimeter. The resulting mount was still thin enough so that a high dry objective brought the top surface of the slide into focus, and permitted the use of an oil immersion objective for the examination of most internal head structures. For volume production, the celluloid may be cut into squares, these placed on top of one another, clamped firmly together, and the desired hole drilled through the resulting block.

Except where noted in the procedure above, liquids should be transferred rather than speci-

mens. This is easily accomplished by using a finely drawn pipette to remove liquids from the container. As the liquid surface recedes, the larvae are stranded on the bottom by gently tipping the container.

Small Stender dishes with covers are convenient containers. These are large enough to accommodate several larvae at a time, yet do not require excessive amounts of fluid. The cover is desirable for identification, to prevent evaporation, and to keep dirt out of the preparation.

A black background was found to be helpful in the determination of the end point of the KOH treatment. Fat bodies may still appear as dense white masses at this stage, but they disappear during subsequent steps in the procedure. If specimens are left longer than 24 hours in KOH, the ends of the hairs begin to curl and the integument is seriously weakened.

Since hairs of larvae mounted in this way retain a high degree of contrast in transmitted light, attempts to stain specimens with acid fuchsin were believed to complicate and prolong the procedure unnecessarily.

From the Communicable Disease Center, Public Health Service, Federal Security Agency, Atlanta, Georgia, and the Department of Parasitology, The Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland.—Richard H. Foote, *Department of Parasitology, The Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland.*

REPORT OF PARASITES FROM THE LAKE SUPERIOR CISCO, *LEUCICHTHYS ARTEDI ARCTURUS*

During the years 1948, 1949 and 1950 two hundred Lake Superior ciscoes were examined for all forms of parasites except intestinal and blood protozoans. Fifty additional fish were examined for the occurrence of two species of parasites only. The fish were obtained during all seasons of the year from commercial fishermen. They were taken in nets off the Minnesota north shore of Lake Superior in the Sucker River area. Fish were examined while fresh and nearly all parasites were alive when fixed. A full account of this work may be found in Warren, Bruce H.—1951—Metazoan Parasites of the Lake Herring, *Leucichthys artedi* LeSeuer 1818 from the North Shore of Lake Superior. M.S. thesis, University of Minnesota Library.

Past literature reporting parasites of *Leucichthys artedi* contains the names of seventeen species. In the present study seven of these species were found as well as three additional species which represent new host records. Every fish studied harbored at least one species of parasite.

Experimental feedings were tried with plerocercoid cysts of *Diphyllobothrium* to determine whether any of them were *D. laruei* Vergeer, 1934. Cysts were fed to five kittens and two puppies with negative results.

A list of the parasites encountered in this study is given below in table 1.

Table 1.—*Parasites encountered in Lake Superior ciscoes*

Parasite	Organ	Fish examined	Fish infected
TREMATODA:			
Crepidostomum farionis Lühe, 1909*	gall bladder	250	3
CESTODA:			
Proteocephalus exiguus LaRue, 1911	intestine	200	154
Eubothrium crassum (Bloch, 1779) Nybelin, 1922	intestine	200	4
Diphyllobothrium oblongatum Thomas, 1946 (plerocercoid)	stomach wall	200	155
Triaenophorous crassus Forel, 1880 (plerocercoid)	flesh	200	41
NEMATODA:			
Cystidicola stigmatura Ward and Magath, 1917	swim bladder	200	144
ACANTHOCEPHAL:			
Neoechinorhynchus cylindratus (Van Cleave, 1913) Van Cleave, 1919*	intestine	200	1
Echinorhynchus leidyi Van Cleave, 1924*	intestine	200	59
COPEPODA:			
Salmincola inermis (Wilson, 1911) Wilson, 1915	gill chamber	200	130
Achtheres coregoni (Smith, 1874) Wilson, 1915	fins	250	17

* New host record.

Acknowledgment is made to Dr. F. G. Wallace, University of Minnesota and Dr. T. O. Odlaug. University of Minnesota, Duluth Branch for help throughout the course of this study.—Bruce H. Warren, *University of Minnesota, Minneapolis, Minnesota.*

AUTOCHTHONOUS INFECTION OF CATTLE IN HAWAII WITH *FASCIOLA HEPATICA* LINN.

The examination of a group of 45 adult flukes recovered from the bile ducts of a cow slaughtered in Honolulu on December 12, 1951, revealed that one fluke was *Fasciola hepatica* (Fig. 1) and the others *F. gigantica.* The *F. hepatica* specimen was suspected because of its

unusual small size in comparison with the others and presence of many eggs in the uterus. It measured as follows: length, 16.5 mm.; maximum width, 8 mm.; cephalic cone, 2.3 mm.; diameter of oral and ventral suckers 745 and 936 μ, respectively. Five of the fully developed eggs in the uterus ranged from 130 to 137 μ in length and 81 μ in width. One of the major morphological differential characters of these two species of flukes is the size of eggs. In *F. hepatica* they are from 130 to 150 μ long by 60 to 90 μ wide, and in *F. gigantica* they are from 156 to 197 μ long by 90 to 104 μ wide. Furthermore, according to observations of the writer, juvenile flukes of *F. gigantica* measuring less than 26 mm. in length usually do not show eggs in the uterus. The cow from which the above fluke specimens were recovered was 22 months when slaughtered; it was born near Kaneohe on the island of Oahu and had been raised in and around a swampy area (Kokokahi swamp).

The above findings appear to represent the first proved case of *F. hepatica* in the Hawaiian Islands. In 1937, Alicata and Swanson (J. Parasitol., **23**: 106–107) reported that *F. gigantica* was the common liver fluke of cattle in Hawaii. Before 1937, however, various authors, including A. Lutz (1892, Centralbl. Bakt. **11**: 783–796) and M. C. Hall (1936, Rev. de Parasitol. **2**: 367–383), had reported that *F. hepatica* was the common liver fluke in this locality. However, in re-examination of fluke specimens in collections which dated back to 1892, all turned out to be *F. gigantica* (Alicata, 1938, Hawaii Agr. Expt. Sta. Bul. 80, 22 pp.; Alicata, 1947, Pacific Sci. **1**: 69–84).

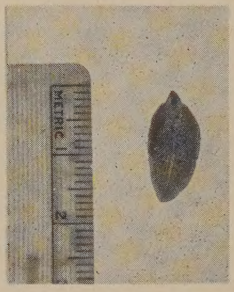

FIG. 1. Young adult *Fasciola hepatica* from cattle in Hawaii.

According to the above information, it appears that the introduction of *F. hepatica* in Hawaii is of comparatively recent origin. In this connection the writer reported (1947, Hawaii Agr. Expt. Sta. Rpt. 1944–46: 99) that in 1944 a cow imported from California and slaughtered in Honolulu 2 months later was found to harbor adult flukes of *F. hepatica*. The local lymnaeid snails, *Fossaria ollula* (Gould), were later infected with miracidia obtained from eggs of these flukes. Rabbits were subsequently experimentally infected with *F. hepatica* by feeding the encysted stage of cercariae emerging from these snails. It was then pointed out that, given an opportunity, *F. hepatica* could become established in the Hawaiian Islands.—JOSEPH E. ALICATA, *University of Hawaii Experiment Station, Honolulu.*

THE INCIDENCE OF COCCIDIA, HEARTWORMS, AND INTESTINAL HELMINTHS IN DOGS AND CATS IN NORTHERN NEW JERSEY

Much information is available as to the incidence of coccidia, heartworms and intestinal helminths in dogs and cats obtained from various localities in the United States. However, as we have been unable to find similar data as to the frequency of occurrence of these particular parasites in small animals in the northern New Jersey area, it was deemed advisable to report the following information.

In the present investigation a total of 100 cats and 55 dogs, all mixed breeds, were secured dead from various sources. The entire gastro-intestinal tract, heart, aorta, and lungs were thoroughly searched for the presence of adult worms. Whenever possible, blood samples were checked by the concentration method of Stubbs and Live (1938, Jour. Amer. Vet. Med. Assn., 92: 686–690) for microfilariae. Fecal examinations were made on a mixture of both caecal and intestinal contents using the saturated-saline centrifuge method. No attempt was made to differentiate between the various species of *Isospora* or *Dipylidium* encountered.

The results show that 85.5 per cent of the dogs and 68.0 per cent of the cats harbored either helminth or protozoan parasites. Furthermore, *Dirofilaria immitis* was found in three of the dogs (5.4%) whereas this parasite was not observed in any of the cats examined. The various species of parasites encountered are shown in Table 1.

TABLE 1.—*Parasites found in 100 cats and 55 dogs in northern New Jersey*

Parasite	Dogs		Cats	
	No positive	%	No positive	%
Ancylostoma caninum	8	14.5	16	16.0
Coccidia (all species)	1	1.8	7	7.0
Dipylidium sp.	17	30.9	19	19.0
Dirofilaria immitis	3	5.4	..	...
Taenia pisiformis	2	3.6	..	...
Taenia taeniaeformis	..	...	8	8.0
Toxascaris leonina	3	5.4	5	5.0
Toxocara canis	8	14.5	..	...
Toxocara cati	..	...	50	50.0
Trichuris vulpis	28	50.9	..	...

It is evident that multiple parasite infections are quite common in these animals. Although the majority of the parasitized dogs and cats harbored either one or two species of parasite, eight of the cats had triple infections and one dog intestine was found to contain *Trichuris vulpis, Dipylidium* sp., *Toxascaris leonina,* and *Taenia pisiformis.*—PHILLIP H. MANN AND ITALO FRATTA, *Dept. of Animal Care, College of Physicians and Surgeons, Columbia University, New York City.*

MYOTIS LUCIFUGUS LUCIFUGUS; A NEW ABERRANT HOST FOR THE THIRD STAGE LARVA OF *PHYSOCEPHALUS SEXALATUS* (MOLIN, 1860).

The natural occurrence of encysted third stage larvae of the spirurid stomach worm of swine has been reported from several aberrant hosts. Among these are the loggerhead shrike, the screech owl, and the red tailed hawk (Cram, 1930, The Auk, 47: 380–384). A natural infection of farm raised chickens was reported by Allen and Spindler (1949, Proc. Helm. Soc. Wash. 16: 1–3). In addition, experimental infections of a wide variety of aberrant hosts has been demonstrated by Cram (*loc. cit.*). Host records of the occurrence of this larval form in species of European bats are: *Myotis dasycneme* (Boie 1825), *Eptesicus serotinus* (Schreber 1774), and *Vespertilio murinus* (Linn. 1758). In the United States, Alicata (1931, J. Parasit. 18: 47) described encysted *Physocephalus sexalatus* from the stomach and mesenteries of *Eptesicus fuscus fuscus* (Beauvois 1796).

Recently the writer had occasion to examine a large series of *Myotis lucifugus lucifugus* (LeConte 1831). In 18 out of 100 bats examined, encysted third stage larvae of *P. sexalatus* were found. The capsules were usually deeply embedded in the duodenum or less frequently were found on the mesenteries. The bats examined were taken from caves in Pendleton Co. West Virginia.—F. G. TROMBA, *Dept. of Zoology, University of Maryland.*

NOTES ON *LEUCOCYTOZOON ANDREWSI* FROM THE DOMESTIC CHICKEN

During the course of investigations in South Carolina concerned with the transmission of this blood parasite, a method was sought to produce parasitemias heavier than the light natural infections ordinarily observed.

Six infected fowls were obtained from their owners and housed in a screened pen. Frequent examinations, usually at weekly intervals, were made of blood films from these chickens for several months and the observations on 4 of them extended 1½ years. Mature gametocytes were noted throughout the period and such variations in numbers as occurred were considered insignificant. Continuous light in the pen for many months had no marked influence on the infection, nor did the onset of the laying period.

At different times from early spring until late fall, 3 of the infected hens and their recently hatched chicks were confined in an open coop at a farmhouse in the experimental area. None of these chicks was found infected for many months thereafter. The effect of splenectomizing one of the experimental hens at a later date did not produce any change in the level of the parasitemia during the following month.

In the original survey of chickens, mature birds were sampled, but in a similar study the next year, emphasis was placed on young fowl. Several different flocks were examined repeatedly throughout the season and a total of 196 immature chickens was observed in association with 54 grown birds. Of the old chickens, 20 were positive for *L. andrewsi* in addition to 4 young fowl in which the organism was found. The nature of the infections in the juvenile birds seemed identical with those of mature fowl in that the parasitemias were light. No organisms were observed in occasional films from individuals known to be infected.

Laboratory trials were performed along with the observations regarding transmission in the field. Approximately 50 young chicks were inoculated intraperiotoneally with either citrated

blood or saline suspensions of comminuted liver, spleen, heart, and lung from infected fowl. Blood films from the young chicks were examined for at least 30 days and in some instances for 60 days with no detectable evidence of *L. andrewsi*.

Numerous lots of mosquitoes were allowed to feed on naturally infected chickens and dissected from 1 day to 3 weeks later. No oocysts or sporozoites were observed in 309 female *Anopheles quadrimaculatus* (Q-1 laboratory strain), 25 *Anopheles crucians*, and 98 *Aedes aegypti* following the blood meals. Fifty specimens of *Culex quinquefasciatus* as well as 6 *A. quadrimaculatus* (Q-1) were comminuted in normal saline several days after feeding on infected chickens. The suspensions were inoculated intraperitoneally into 7 young chicks with no successful transfers noted. A few *C. quinquefasciatus* and *A. quadrimaculatus* (Q-1) were allowed to bite an infected fowl and later permitted to feed on 3 young chicks. The chicks remained apparently negative for *L. andrewsi* the following month.—FLOYD O. ATCHLEY, *Communicable Disease Center, Public Health Service, Atlanta, Georgia, and State Board of Health, Columbia, South Carolina.*

DEMONSTRATION OF RIBONUCLEIC ACID IN THE ONCHOSPHERE AND ADULT OF *MESOCESTOIDES* BY MEANS OF RIBONUCLEASE

Entire tapeworms and gravid proglottids identified as *M. variabilis* were fixed alive in Zenker's fluid and acetic acid-formalin-alcohol mixture, embedded in 62° tissuemat and sectioned at 10 microns. This cestode tissue was stained with methyl green-pyronin as prepared by Vogel (1930, Zeit. Parasitenk., 2: 213–222). Sections were stained five minutes, then dipped in acetone quickly, cleared in cedar wood oil, placed in xylol and mounted in piccolyte. This procedure provided a beautiful red and green preparation. The pyronin stained the cytoplasm of the subcuticular cells, muscle cells, reproductive cells, uterine cells and parenchymal cells, of the adult, a bright red. The embryonic plastin, or germinative cells of the onchospheres also showed an intense red coloration of the cytoplasm. According to various authors and more recently Kurnick (1950, J. Gen. Physiol., 33: 243–264) it has been demonstrated that the pyronin acts as a relatively specific stain for ribonucleic acid. Methyl green has been considered a specific stain for desoxyribonucleic acid. The cytoplasm of the cestode cells stained red with pyronin. The nucleus stained green with methyl green.

In order to demonstrate that the pyronin was staining ribonucleic acid, a specific enzyme was required to remove the stainable substrate. Crystalline ribonuclease, salt free, obtained from Worthington Biochemical Sales Co. was dissolved in the proportion of 1 mg./ml. of acetate-veronal buffer of pH 6.7 made according to Michaelis (1931. Biochem. Zeitschr., 234: 39). The experimental procedure of Opie and Lavin (1946, J. Exptl. Med., 84: 107–112) was employed. Special glass slides were prepared by cementing a piece of glass across each end. Moist chambers were made from petri dishes. The bottom was covered with moist filter paper. Two wooden applicator sticks served as supports for the slides. Zenker and AFA fixed sections were used. Tissuemat was removed by xylol and the sections were hydrated. Zenker material was treated with iodine, washed in 70 per cent alcohol, then two changes of distilled water. The special slides with glass supports each received 0.3 ml. of solution. Test slides with sections were inverted over the drop so that contact was made and all tissue was covered. Two control slides received buffer only. Three test slides received the ribonuclease in the buffer. All preparations were placed in the moist chambers and incubated for 2 hours at 37° C. After incubation all slides were removed, washed in distilled water and stained.

Methyl green-pyronin was used to stain all sections. Control sections, treated only with buffer, stained normally and showed the pyronin red coloration for ribonucleic acid in the cytoplasm of subcuticular cells, muscle cells, parenchymal cells, uterine cells, and the majority of observable cells in the proglottis as well as the granules in the parenchyma. The cytoplasm of the plastin cells of the onchosphere and early embryo also stained a deep red and the nucleus was green. Sections treated with ribonuclease in the acetate-veronal buffer at pH 6.7, however, showed the complete absence of the pyronin red coloration in the parenchyma. However, a small amount of very faint dispersed pink to red coloration showed in the subcuticula in irregular patches in some sections. The greater density of the subcuticula and the greater concentration of stainable substance explains this in part. Longer treatment with ribonuclease would probably have removed all stainable material. The plastin cells of the onchospheres and the earlier embryos also failed to stain red with pyronin. The nuclei, however, stained green with methyl green. Comparison of controls with ribonuclease-treated sections clearly demonstrated that the above enzyme concentration acting for two hours removed most of the pyronin stainable material. If the ribonuclease was a specific enzyme for the ribonucleic acid under the conditions of this experiment, then the pyronin stainable material was ribonucleic acid.—ROBERT E. OGREN, *Zoology Department, University of Illinois, Urbana, Illinois.*